BIOCHEMISTRY OF THE FATTY ACIDS

AND THEIR COMPOUNDS, THE LIPIDS

By
W. R. BLOOR
Professor of Biochemistry and Pharmacology,
The University of Rochester,
Rochester, New York

American Chemical Society
Monograph Series

REINHOLD PUBLISHING CORPORATION
330 WEST FORTY-SECOND STREET, NEW YORK, U. S. A.
1943

Second Printing

Printed in the United States of America by
THE GUINN CO., N. Y. C.

GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic monographs on chemical subjects. At the same time it was agreed that the National Research Council, in coöperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, secretary of the society, Washington, D. C.; the late John E. Teeple, then treasurer of the society, New York; and Professor Gellert Alleman of Swarthmore College. The Trustees arranged for the publication of the A. C. S. series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company, Inc. (Reinhold Publishing Corporation, successors) of New York.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed editors (the present list of whom appears at the close of this introduction) to have charge of securing authors, and of considering critically the manuscripts submitted. The editors endeavor to select topics of current interest, and authors recognized as authorities in their respective fields.

The development of knowledge in all branches of science, especially in chemistry, has been so rapid during the last fifty years, and the fields covered by this development so varied that it is difficult for any individual to keep in touch with progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and by such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie, Moissan's Traité de Chimie Minérale Générale, Friend's and Mellor's Textbooks of Inorganic Chemistry and Heilbron's Dictionary of Organic Compounds, it often takes a great deal of time to coördinate the knowledge on a given topic. Consequently when men who have spent years in the study of important subjects are willing

to coördinate their knowledge and present it in concise, readable form, they perform a service of the highest value. It was with a clear recognition of the usefulness of such work that the American Chemical Society undertook to sponsor the publication of the two series of monographs.

Two distinct purposes are served by these monographs: the first, whose fulfillment probably renders to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a form intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs enable such men to form closer contact with work in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well-digested survey of the progress already made, and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, extended references to the literature enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection is made of those papers which are most important.

Preface

Proteins, carbohydrates and fats constitute the great groups of organic food substances. Of these, the proteins and carbohydrates have received much attention from biochemists, while the fats, or more specifically the fatty acids and their compounds, are only now passing through a similar stage of development.

In view of the increasing interest in these substances, which are now recognized as taking a part in living structures and processes second only to the proteins, it is desirable to gather together and evaluate the reported material so that it may serve as a background for future effort. The following is an attempt in this direction. A clear picture of the various aspects of the field is not possible at the present time, but it is hoped that what is presented will make such a picture possible as the new facts appear.

The bibliography has presented the usual difficulty. Limits of space prohibit the listing of all or even the more significant publications. What is given is a list of those articles which either contribute most to the general discussion or contain references which lead the interested reader to other material not specifically mentioned.

W. R. Bloor.

Rochester, N. Y.
April 15, 1943.

Contents

General Introduction iii
Preface v

Chapter I. Chemistry: Descriptive and Analytical

Classification of the Lipids 1
Simple Lipids 3
Fats 3
Constitution 3
Physical Properties 4
Chemical properties 8
Synthesis 10
Waxes 11
Compound Lipids 12
Phospholipids 12
Lecithin 12
Cephalin 16
Lysolecithin and lysocephalin 17
Sphingomyelin 18
Phosphatidic acids 19
Glycolipids (galactolipids or cerebrosides) 19
Sulfolipids 20
Derived Lipids 21
Fatty acids 21
Occurrence 21
Saturated acids 21
Unsaturated acids 21
Properties of the fatty acids 25
Alcohols in natural combination with the fatty acids 30
Glycerol [$C_3H_5(OH)_3$] 30
Straight-chain alcohols 31
The sterols 31
Cholesterol 32
Bases 35
Hydrocarbons 36
Split products 36
Physico-chemical Conceptions of the Lipids 36
Methods of Examination of Tissue Lipids 37
Macromethods 37
Discussion 37
Hydrolysis 38
Dehydration and extraction 38
Procedure 40
Phospholipids 42
Cholesterol 42
Cholesterol esters 43
Fats 43
Fatty acids 43
Micromethods 44
Illustrative procedure 45
Extraction 45
Isolation 46
Discussion 49

CHAPTER II. DIGESTION AND ABSORPTION

Nutritional Availability of Fats 58
Factors influencing digestion and absorption 59
Melting point 59
Nature of the fatty acids 61
Temperature 61
Vitamins 62
Species variation 62
Lipid-splitting Enzymes 62
Lipases (Fat-splitting Enzymes) 66
Gastric lipase 66
Pancreatic lipase 67
Intestinal lipase 70
Plant lipase 70
Methods of measuring activity 72
Esterases (Ester-splitting Enzymes) 73
Origin 73
Occurrence 74
Functions of esterases 77
Fat Digestion 78
Digestion in the Stomach 79
Factors in fat splitting 79
Absorption from the stomach 80
Intestinal Digestion 81
Passage from the stomach 81
Factors in intestinal digestion 82
Absorption of Fats 85
Form in which Fat Leaves the Intestine 85
Absorption of split products 85
Absorption of unchanged fat 87
Resynthesis of Fat 91
Fat Transport 95
Transfer to the lacteals 95
The lymph in fat transport 97
Changes in Fat During Absorption 98
Passage of Fat Directly into the Circulation 100
Parenteral Administration of Fat 103
Digestion and Absorption of Other Lipids 103
Phospholipids 103
Sterols 104
Cholesterol 104
Other sterols 108

CHAPTER III. LIPIDS OF THE BLOOD

Introduction 115
Blood as a tissue 115
Nature of the fatty substances of blood 116
Blood lipid equilibrium: the postabsorptive state 118
Lipid level in the postabsorptive state 119
Factors affecting the lipid level 120
Normal Basal Levels of Blood Lipids 122
Average Values 122
Data on different species 122
Lipid "constants" 128
Normal Variations in Members of the Same Species 129
Normal Variations in the Same Individual 131
Human Blood 132

Changes in the Postabsorptive Level Produced by Food ... 136
Effect of Fat Absorption on the Blood Lipids ... 136
Effect on fat ... 136
Effect on cholesterol ... 137
Effect on phospholipid ... 139
Fat ingestion and the blood lipids in humans ... 140
Factors Modifying the Effect of Fat Absorption ... 141
Removal mechanism ... 141
Lipid destruction in the lungs ... 142
Nutritional state and diet ... 143
Intravenous Injections ... 144
Effect of Diet on the Blood Lipids ... 145
Variations in Blood Lipids in Normal Individuals ... 147
The Reproductive Cycle and the Blood Lipids ... 147
Age ... 151
Work ... 152
Effect of Abnormal Conditions on the Blood Lipids ... 153
Nutritional Abnormalities ... 153
Fasting ... 154
Anesthesia and narcosis ... 156
Anemia ... 158
Cancer ... 162
Vitamin deficiencies ... 163
Avitaminosis ... 163
Vitamin B deficiency ... 163
Scurvy ... 164
Vitamin A deficiency ... 164
Irradiation ... 164
Magnesium deficiency ... 164
Diabetes ... 164
Insulinization ... 167
Effect of various drugs ... 168
Phlorizin ... 168
Phosphorus ... 168
Hydrazine poisoning ... 169
Acids ... 169
Snake root or rich weed (Eupatorium verticaefolium) ... 169
Tartrates ... 169
Deposits of metabolic excesses ... 169
Muscular dystrophy ... 170
Fattening and obesity ... 170
Epilepsy and water balance ... 170
Skin disorders ... 171
Infections ... 172
Infections and immunity ... 172
Serological reactions ... 173
Tuberculosis ... 174
Leprosy ... 175
Syphilis ... 175
Mental Disease ... 175
Organic Diseases ... 175
Angina pectoris ... 175
Arteriosclerosis and hypertension ... 175
Arthritis ... 177
Liver disease ... 177
Kidney disorders ... 179
Pancreatitis ... 181
Thyroid conditions ... 181
Hypophysis dysfunction ... 182
Operative procedures ... 182

Effect of the Lipids of the Blood on its Properties ... 183
Lipemia ... 183
Embolism ... 183
Hemolysis ... 183
Coagulation ... 184
Sedimentation time of corpuscles ... 184
Physico-chemical relations ... 184
Combinations of the lipids with other substances in blood ... 185
Summary ... 186
Blood lipid composition in normal animals ... 186
Abnormal bodily states ... 187

Chapter IV. The Lipids in Tissue

Introduction ... 196
Data on Tissue Lipids ... 198
Muscle ... 198
Normal variations ... 198
Muscle lipids under various abnormal conditions ... 202
Brain and nervous system ... 203
Liver ... 208
Kidney ... 219
Adrenal glands ... 219
Spleen ... 221
Bone marrow ... 222
Reproductive organs ... 222
Skin and appendages ... 225
Gall bladder ... 228
Human fat ... 228
Cold-blooded animals ... 228
Tissue Lipids in Abnormal Conditions ... 229
Diseases involving lipid deposits ... 231
Nature and Function of Tissue Lipids ... 236
Fat ... 236
Nature of stored fat ... 236
Effect of environmental temperature on nature of stored fat ... 240
Phospholipids and Sterols ... 244
Role as essential cellular constituents ... 244
Fatty acids in combination in the phospholipids ... 252
Relation of lipid content to physiological activity ... 256
Lipids of Plants ... 257
Fat ... 257
Phospholipid ... 258
Unsaponifiable ... 260
Lipids of Microorganisms ... 263
Bacteria ... 264
Yeast ... 273
Molds ... 275

Chapter V. Lipid Metabolism

Introduction ... 286
Physiological Synthesis of the Lipids ... 287
Synthesis of Neutral Fat ... 287
Synthesis of Sterols ... 291
Synthesis of Phospholipids ... 293
Intermediary Metabolism and the Role of the Liver ... 294
Fat Deposition and Mobilization ... 294
Mobilization and storage in the liver ... 294
Phospholipids of the liver in fat metabolism ... 300

The change of fat to carbohydrate ... 302
Obesity ... 308
Changes in the migrating salmon ... 309
Functions of the Lipids in the Body ... 310
Fat ... 310
Phospholipids ... 312
Cholesterol ... 313
Catabolism of the Fats ... 313
Oxidation Mechanisms for Neutral Fats ... 313
Unsaturation ... 314
Desaturation as a stage in fatty acid metabolism ... 314
Hydrogenation or saturation ... 317
The need of unsaturated fatty acids by the organism ... 317
Fat deficiency; essential fatty acids ... 318
Relative value of fatty acids in nutrition ... 320
Oxidation at the double bond ... 320
β-Oxidation ... 322
Multiple oxidation and fragmentation ... 325
Summary ... 326
Ketogenesis and Antiketogenesis ... 326
Formation of ketone bodies (ketogenesis) ... 327
Acid intoxication ... 330
Removal of ketone bodies (antiketogenesis) ... 330
Effect of High-Fat Diet ... 336
Metabolism of Cholesterol ... 336
Fat Metabolism in the Developing Embryo ... 337
The Vitamins in Lipid Metabolism ... 342

CHAPTER VI. THE LIPIDS OF SECRETIONS AND EXCRETIONS

Secretions ... 350
Food for the embryo and young organism ... 350
The placenta ... 351
Milk ... 352
Eggs ... 359
Lymph and chyle ... 363
Cerebrospinal fluid ... 363
Bile ... 364
Excretions ... 366

Chapter I

Chemistry: Descriptive and Analytical

Development of the Term "Lipid"

Most of the organic substances which go to make up plant and animal tissues fall into three great groups. These are sharply defined and do not overlap except in unimportant particulars. The groups are: the proteins, with the amino acids as their characteristic constituents; the carbohydrates, with the monoses as the units; and the fatty substances, the chemical entity characterizing which is the fatty acid, the members of the last group being either actually or potentially compounds of the fatty acids. Various names have been proposed for this group, but so far none has been universally accepted. The most recent term, "lipid" (or "lipide"), is the one recommended by the International Congress for Pure and Applied Chemistry and will be used in the present discussion as the general term for the group. The name recommends itself for the following reasons: (a) its derivation is suitable; (b) it is general enough to include the diverse members of the group; and (c) it has not been used for any of the subgroups, while the other terms proposed have been so used. Leathes and Raper (1925), in their monograph, use the term "fats" as the general term, although it is in common use for one division of the group: the ordinary triglycerides of the fatty acids, the neutral fats. "Lipin", suggested by Rosenbloom and Gies (1912), has since been used by the MacLeans (1927) in their monograph for another division of the group. The term "lipoid" has always been used to include all members of the group except fats: "fats and lipoids."

Classification of the Lipids

Lipids may be defined as a group of naturally occurring substances consisting of the higher fatty acids, their naturally occurring compounds, and substances found naturally in chemical association with them. The group is characterized in general by insolubility in water and solubility in "fat solvents", *e.g.*, ether, chloroform, benzene, etc. It may be divided as follows:

Simple lipids: esters of the fatty acids with various alcohols.

Fats: esters of the fatty acids with glycerol. (Fats which are liquid at ordinary temperatures are called oils.)

Waxes: esters of the fatty acids with alcohols other than glycerol. Examples: beeswax, lanolin, cholesterol palmitate.

Compound lipids: compounds of the fatty acids with alcohols, but containing other groups in addition to the alcohol.

Phospholipids (phosphatides): substituted fats containing phosphoric acid and a nitrogen compound, *e.g.*, lecithin, cephalin, sphingomyelin.

Phosphatidates: phospholipids minus the organic bases.

Glycolipids: compounds of the fatty acids with a carbohydrate and a nitrogen compound but containing no phosphoric acid, *e.g.*, the cerebrosides.

Sulfolipids: lipids containing sulfuric acid.

(*Aminolipids*, etc.: possible groups which are as yet not sufficiently well characterized.)

Derived lipids: substances derived from the preceding group which have the general properties of the lipids.

Fatty acids of various series.

Alcohols, including glycerol: mostly large molecular solids found naturally in combination with the fatty acids and soluble in fat solvents. Examples: cetyl alcohol ($C_{16}H_{33}OH$), myricyl alcohol ($C_{30}H_{61}OH$), and cholesterol ($C_{27}H_{45}OH$).

Hydrocarbons: compounds such as squalene which in some animals (sharks) accumulate in the liver.

Bases: choline, aminoethyl alcohol, sphingosine.

For the present discussion, the group of lipids is specifically limited and defined in two ways: (a) only substances are included which are chemically and metabolically related to the fatty acids; (b) only naturally occurring substances are included.

The definitive chemical entity is the fatty acid, hence the title of the monograph; and the group of lipids is intended to include only those substances which are closely concerned with the biochemistry of the fatty acids. The second limitation, "naturally occurring", is intended to exclude organic compounds which have no relation to the use of the fatty acids by living things, but which would otherwise be included because of composition or physical properties. Such substances would be the mineral oils, essential oils, etc.

For more detailed discussion of the facts presented in this monograph, the reader is referred to the following monographs: Halden and Grün (1929), "Analyse der Fette und Wachse"; Leathes and Raper (1925), "The Fats"; MacLean and Smedley-MacLean (1927), "Lecithin and Allied Substances, the Lipins"; Jamieson (1932), "Vegetable Fats and Oils";

Thierfelder and Klenk (1930), "Die Chemie der Cerebroside und Phosphatide"; Bull (1937), "The Biochemistry of the Lipids"; and Sobotka (1938), "The Chemistry of the Sterids".

SIMPLE LIPIDS

Fats

The esters of the fatty acids with glycerol are commonly called oils when they are liquid at ordinary temperatures and fats when they are solid. They are the most important of the lipids from the point of view of quantity, wideness of distribution, food value, and commercial interest. They constitute the main form of food storage in animals, sharing this function with carbohydrates and, to a less extent, with proteins. As they occur naturally, they are always mixtures of triglycerides of various fatty acids, and their properties vary with the nature of the glycerides composing the mixture.

Constitution. The glycerides may be either simple or mixed, as shown by the following formulas where *R* is a fatty acid, either saturated or unsaturated:

Simple glyceride	*Mixed glycerides*		
$CH_2.OOC.R$	$CH_2.OOC.R$	$CH_2.OOC.R$	$CH_2.OOC.R$
|	|	|	|
$CH.OOC\ R$	$CH\ OOC.R_1$	$CH.OOC.R_1$	$CH.OOC\ R$
|	|	|	|
$CH_2.OOC.R$	$CH_2\ OOC\ R$	$CH_2.OOC.R_2$	$CH_2\ OOC.R_1$

Far from being rare constituents of the natural fats, as was first believed, more recent investigations tend to show that mixed glycerides form the bulk of many of the natural fats. Of the mixed glycerides, some have possibilities of optical activity (see discussion under "Optical Activity", p. 8). It should be noted that the phospholipids, lecithin and cephalin are naturally occurring mixed glycerides containing, generally, two different fatty acid radicals.

The nature of the glyceride mixtures which are found in naturally occurring fats has been given considerable attention in recent years, especially by Hilditch and his associates (Collin and Hilditch, 1929; Hilditch, 1930; Banks and Hilditch, 1931) and also by Lovern (1936), and their work leaves little doubt that mixed glycerides are probably the main constituents of natural fats. They find also that, given the usual variety of fatty acids, the nature of the mixed glycerides will depend to a considerable extent on the type of organism producing the fat and on the environment, although the main determining factor is the nature of the fatty acid mixture. Hilditch's findings may be summed up briefly as follows: Simple triglycerides are found in quantity in the

natural fats only when no other form of combination is available. Animal fats are more heterogeneous than vegetable fats (other than linseed oil which is likely to resemble animal fats). Seed fats are mainly mixed glycerides with only a few simple glycerides, and the unsaturated fatty acids tend to be uniformly distributed throughout the fat. Animal fats and fats from the pericarp of vegetables have more saturated glycerides. Solid vegetable fats consist mainly of mono-oleo-di-saturated glycerides with a few di-oleo-mono-saturated glycerides. When unsaturated acids predominate in animal fats, more than 30 per cent of the stored material must be present as mixed glycerides before any saturated triglycerides can be formed. On the other hand, if the *total saturated/unsaturated* acids ratio is greater than 2:1, triolein is absent. No triolein is present in coconut or palm nut oil. The difference between animal and seed fats is well shown by comparing two seed fats, cocoa butter and illipe fat, with cow butter and mutton tallow. In the animal fats, there is present 26 to 31 per cent of saturated triglycerides, and in the seed fats, only 2.5 to 4.5 per cent, although the content of unsaturated acids is nearly the same in all four fats. Typical of higher animal fats is the following (Hilditch and Paul, 1938). Ox depot fat in molar per cent was as follows: oleopalmitostearin 32, palmitodiolein 23, oleodipalmitin 15, stearodiolein 11, and saturated glycerides, mainly dipalmitostearin and palmitodistearin, 17. Very small amounts of tripalmitin and tristearin were found. Triolein was absent, or present only in traces, with traces of myristic, hexadecenoic and arachidic acids. Stearoglycerides result from hydrogenation of oleoglycerides.

Marine fats consist of a complex mixture of fatty acids of varying molecular size, but mainly unsaturated, but land animal fats are much simpler. Among the land animals, there appear to be two groups: (1) birds and rodents with 25-30 per cent palmitic acid and with the fatty acids evenly distributed as in plants; and (2) pig, ox, sheep, and other herbivorous animals, in the depot fat of which stearic acid has become more important and the amount of saturated glyceride much greater. There is a gradual simplification in fatty acid composition from aquatic fats to fats of the higher land animals.

Physical properties. *Solubilities.* The glycerides of the higher fatty acids are insoluble in water; those of the lower fatty acids, *e.g.*, butyric, are somewhat soluble. In organic solvents such as ether, chloroform, and benzene, all are soluble, even in the cold, and generally much more soluble in the hot solvents. In ethyl and methyl alcohol and acetone, the glycerides of the higher fatty acids are slightly soluble in the cold, but readily soluble when hot. In fact, boiling ethyl alcohol is one of the best solvents for use in extracting lipids from tissues; it gives, in most cases, a more

complete extraction than ether, chloroform, or benzene, probably because it penetrates the tissue better, owing to its affinity for water. The solubility in alcohol, like the melting point, varies with the nature of the combined fatty acid, the glycerides of the unsaturated and the lower saturated fatty acids being more soluble than those of the higher saturated acids. The glycerides of the hydroxy fatty acids, like the acids themselves, are insoluble in petroleum ether.

Melting point and solidifying point. In general, the melting points of the glycerides are higher than those of the contained fatty acids and vary with the fatty acids, the glycerides of the higher saturated acids having the highest melting points and those of the unsaturated acids the lowest. The melting point of a natural fat, which is always a mixture of glycerides, depends on the nature of the component glycerides. Its melting point may be low by reason of the presence of either glycerides of the lower acids or glycerides of the unsaturated acids. The melting points of mixtures of pure glycerides cannot be foretold from the melting points of the constituents; eutectic mixtures are formed, the melting points of which pass through a characteristic minimum value below that of either of the constituents. On the other hand, having determined the curve of melting points of various known mixtures of pure triglycerides, it is possible to determine by its melting point the composition of an unknown mixture with a fair degree of accuracy, a fact which has been made use of by Twitchell (1914; 1917).

The solidifying point of a glyceride or mixture of glycerides is always lower than the melting point, the difference generally being considerable and often wide. Thus the melting point of tristearin is given as 71.6° and its solidifying point about 52° (Lewkowitsch, 1921). A sample of beef fat (from the heart) melted at 49.5° and solidified at 36° (Abderhalden, 1911), and butter fat melted at 34.5° and solidified at 22.7° Analogous to these findings is the fact that pure triglycerides, under certain conditions, will exhibit a double melting point. Tristearin, for example, which has just been melted will melt at 55°. On raising the temperature, it will solidify and melt again at 71.5° (Guth, 1903).

Since these peculiarities of the glycerides conflict with the physical law that chemical compounds should exhibit constant properties, a considerable amount of work has been done to clear up the inconsistency. It was found by Guth (1903), and Le Chatelier and Cavaignac (1913) that well crystallized tristearin has but one melting point (71.5°). If, however, it was examined shortly after having been melted, it showed two melting points due to a delayed cooling. Le Chatelier and Cavaignac (1913) also showed that, in glyceride mixtures, the change from liquid to solid was extremely slow, and that, if the observations were carried out with

sufficient slowness, the melting and solidifying process reversed within 0.1-0.2°. Brigl and Fuchs (1922) claimed that fatty acids of identical structure might differ in their melting point, depending upon the different orientation of the carbon atoms in the crystal. Bömer (1907) was able to verify the double melting point of tristearin and of various natural fat mixtures, and concluded that the double melting point was due to the presence of two isomeric forms of the glycerides, one labile and the other stable. In rapid cooling, the labile form is present and at the lower melting point it changes over into the stable form. The first melting point is the changing-over point. Grün and Schacht (1907) prepared synthetically three mixed glycerides which could be prepared in either the lower (labile) or higher (stable) melting form. The labile form could be gradually converted into the stable by seeding with a crystal of the stable form, but the reverse change was not possible. The results of these workers seem to indicate the existence of an unknown factor, possibly the occurrence of two crystal forms of the glycerides.

In further support of this conception, Garner and King (1929), from a study of heats of crystallization of the fatty acids, found evidence for at least two crystal forms in both the odd- and even-numbered carbon atom series of fatty acids. Clarkson and Malkin (1934) concluded that simple triglycerides occur in two crystal forms. Wooley and Sandin (1935), using nonadecylic acid, m.p. 68-68.5°, formed triglycerides *a* m.p. 66.5-67°, *b* 70.5°, and *c* 60°, *a* and *b* being crystalline, while *c* was glassy. Grüntzig (1939) showed photomicrographs of polymorphic forms of various triglycerides. Each of the C_{13}, C_{15}, and C_{17} glycerides had four polymorphic forms. The relations between the melting points of the various forms are discussed. From a practical point of view, the rule has originated that to get a true melting point it is necessary for the fat to stand for at least 24 hours in the melting-point tube before the determination is made.

The delayed solidification is of considerable importance in the living animal since, in many herbivorous animals, the stored fats have a melting point considerably above body temperature, while the solidifying point is some degrees below it (note beef fat).[1]

Color, odor, and taste. The pure triglycerides are colorless, odorless and tasteless; the presence of these properties is due entirely to foreign substances mixed with or dissolved in the glycerides. Thus the commercially desirable yellow color of butter is due to plant pigments carried over from the food. Plant pigment is also responsible for most of the

[1] A condition has been noted in infants (*Sclerema neonatorum*) in which the subcutaneous fat hardened, resulting in the death of the infant (Smith, 1918). In this case, the abnormality found was a high content of free fatty acid.

color of the stored fat of animals (Palmer and Eckles, 1914; Palmer, 1922). Palmer and Eckles (1914) in their study of the relation of carotene, xanthophylls, etc., to various colors conclude:

(1) That the fat of cows' milk owes its color to carotene and xanthophyll, mainly carotene. These are not synthesized by the animal but are taken up with the food. The pigment in the body fat may be called on in the absence of suitable food to supply color for the milk fat. All breeds of cows may be put through the changes from colorless to highly colored butter by changes in the feed. Fresh cows give the highest color.

(2) That the lipochrome of body fat, corpus luteum, and skin secretions in the cow is mainly carotene.

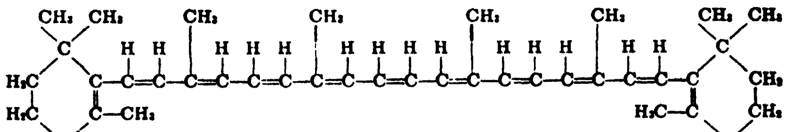
(3) That the lipochrome of blood serum is mainly carotene and is firmly combined with albumin or lecithin and cholesterol.

(4) That carotene is more stable toward gastric juice, etc., than xanthophyll.

(5) That human milk and body fat are colored similarly.

(6) That the greenish yellow color of whey (lactochrome) is probably urochrome.

The nature of these pigments is now well worked out, mainly by the efforts of Karrer in Switzerland, Kuhn in Germany and Zechmeister (1934) in Hungary. The formula for β-carotene, m.p. 182°C., is:



β-Carotene

This compound, split at the central point and with water added, becomes vitamin A, a colorless alcohol. Closely related to carotene chemically is the other common plant pigment, xanthophyll, a dihydroxy alcohol which forms esters with the fatty acids and so becomes soluble in the fat solvents.

The amount of color present is very small. Zechmeister and Tuzson (1934) found in 1 kg. of chicken fat 5 mg. of lipochrome containing 40 per cent xanthophyll. A kilo of horse fat yielded 6 mg., almost entirely β-carotene. Cow fat contained mostly carotenes. The association of color with vitamin A activity is now well recognized, and agents which destroy the color destroy the vitamin activity also (Shrewsbury and Kraybill, 1933; Palmer and Eckles, 1914; Steenbock, Boutwell and Kent, 1920).

The flavor of food fats is due to foreign materials absorbed by the fat, either from its natural environment, or from materials formed during

the processes of preparation. Thus, in modern butter-making, the bacterial flora are carefully controlled with this point in mind.

Optical activity. Certain of the mixed triglycerides should be optically active since they contain an asymmetric carbon atom. Thus:

$$C_3H_5\begin{cases}O.R_1\\ O.R_2\\ O.R_3\end{cases} \text{ and } C_3H_5\begin{cases}O.R_1\\ O.R_1\\ O.R_2\end{cases} \text{ should be optically active}$$

As a matter of fact, the only optically active fats which are found in nature are those which contain optically active fatty acids as, for example, castor oil and chaulmoogra oil, and those which contain optically active nonfat substances, such as resins and sterols (cholesterol, phytosterol, etc.). Attempts have been made from time to time to prepare optically active glycerides (Abderhalden and Eichwald, 1915; Bergmann and Sabetay, 1924) without much apparent success, the reason for which is probably, as pointed out by Bergmann and Sabetay, that the optical activity is too small for measurement. (See also Baer and Fischer, 1939.)

Spectroscopic behavior. Moore and associates (1939) found that the fats with conjugated fatty acids such as in tung oil showed absorption at 270 mμ. With the loss of one conjugated linkage, the absorption was at 230 mμ. Refluxing with potassium hydroxide caused a reconjugation. Miller and Burr (1937) have made use of these labeled fatty acids in studies on fat metabolism.

Chemical properties. *Hydrolysis* (saponification). Fats are hydrolyzed in the same way and with the same agents as are simple esters. Water at high temperatures, as in an autoclave, may be used either alone or, more advantageously, with catalysts such as acids or alkalies. At ordinary pressures the same catalysts do the work, but more slowly. The speed of reaction may be increased by the use of a solvent, such as alcohol, which dissolves both the fats and the catalyst. Amyl alcohol is more effective than ethyl alcohol, probably because of the higher temperature which can be obtained. Alkali may be added to the alcohol as such or, more effectively, in combination with the alcohol as alcoholates.

Fat-splitting enzymes, or lipases, are present in the gastrointestinal secretions of animals, and in many plants, especially in fatty seeds such as the castor bean and the seeds of *Chelidonium majus*, the rubber plant, etc. Their use and behavior will be considered in the chapter on digestion (Chapter II).

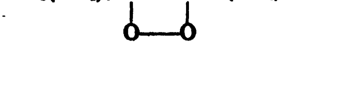
The term "saponification" (by derivation, soap formation), is loosely used as equivalent to hydrolysis. Such a use is misleading, and the term should be confined to such processes as involve actual soap formation, *i.e.*, where metallic bases are used for hydrolysis.

Rancidity. Rancid fats are regarded as unfit for food, partly because of the disagreeable taste and smell, and partly because they appear to be mildly poisonous to some individuals. The possible poisonous effects of rancid lard on rats have been studied by Powick (1925), who found that it was not directly toxic, but that vitamin A was destroyed by the activity of the organic peroxides. Lease and associates (1938) found that rancid fats, ozonized fats, and palmitic peroxide destroy vitamin A and that rancid fats destroy carotene. Addition of antioxidants does not prevent the destruction. The oxidation products of glycerol, on the other hand, do not destroy vitamin A.

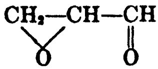
In general, workers are agreed that rancidity is the result of oxidation by atmospheric oxygen in the presence of moisture and especially of light and heat. Metals in small amounts appear to catalyze the process (Emery and Henley, 1922; and Greenbank and Holm, 1924). Dry air, when light is excluded, has apparently little effect on oils and fats. Unsaturated fats become rancid more quickly than saturated. In fact, most investigators are of the opinion that oleic acid is the acid most concerned in rancidity (Holm and Greenbank, 1924), linoleic and linolenic being less important.

Holm and Greenbank (1923; see also Greenbank and Holm, 1924, 1925; and Holm, Greenbank and Deysher, 1927) have discussed the catalytic action of light and other factors in the oxidation of fats and oils in the presence and absence of free oxygen. Changes in the chemical constants of the fat and reaction products were used as criteria. In the absence of free oxygen, light caused slow oxidation with a decrease in the weight of the fat and the production of carbon dioxide and water, the fats becoming exceedingly tallowy in flavor. There was always an induction period in the rancidity process which was prolonged by moisture and shortened by ultraviolet light. Hydroxyl compounds retarded rancidity, unsaturated fatty acids catalyzed it, and peroxides allowed oxidation even in a vacuum. During the auto-oxidation, the fat-soluble vitamins were destroyed.

The evidence points to the double bond as the point of attack by oxygen in rancidity; the products obtained and the characteristic reactions of rancidity may be referred to changes, of which the action of oxygen at the double bond is the earliest. The stages in the process have been outlined as follows by Kerr and Sorber (1923): first, the development of free acid; then, a drop in free acid, followed again by increased acidity; finally, a fall in the iodine value, an increase in unsaponifiable matter, and a fixation of oxygen in a peroxide form of the following nature:



This substance acts as a carrier of oxygen for the formation of various oxidation compounds, the nature of which is not well known. Powick's work (1923) has eliminated a large number of possibilities and indicates that heptylic and nonylic aldehydes are largely responsible for the odor, and that the color obtained in the Kreis test is due to a substance of the nature of epihydrin aldehyde.



Holm and Greenbank (1923) found that the intensity of the Kreis rancidity test was directly proportional to the amount of oxygen absorbed; also that fats would sometimes give a strong Kreis test without showing other evidence of rancidity. Kerr and Sorber (1923) emphasize the presence of labile oxygen in the rancid fat.

Cummings and Mattill (1931) state that among the products of rancidity are free fatty acids, aldehydes, ketones, and peroxides. Evidence from acetyl number determinations has shown the presence of hydroxyl groups. Methylamyl, methylnonyl, and methylheptyl ketones are mentioned by Stokoe (1928), and methylalkyl ketone by Fierz-David (1925), as probably present in rancid fats.

The use of hydroxy aromatic compounds as antioxidants and hence as factors in rancidity production was investigated by Mattill (1931), who found that, in the case of the phenols, the ortho- and para- compounds were active, but the meta- compound was not. The hydroxyl group must be directly attached to the ring. Inosite was inactive. In the naphthols, one hydroxyl group was effective; quinone was ineffective, as were also animal and plant sterols.

Synthesis. Both simple and mixed glycerides have been prepared synthetically in considerable variety (Grün and Theimer, 1907). The methods of synthesis of the mixed compounds are not markedly different from those for the simple glycerides, but the procedure is somewhat complicated by the fact that glycerol is a triatomic alcohol with two different positions, and that there may be shifting of the groups from one position to the other. For example, Kreis and Hafner (1903) found that when oleic acid was allowed to act on dipalmitin and distearin, considerable quantities of tripalmitin and tristearin were found, while the yields of oleodipalmitin and stearin were correspondingly reduced. The special problems involved are reviewed by Amberger and Bromig (1922) and by Daubert and King (1939). Verkade, van der Lee and Meerburg (1932) heated glycerol with a slight excess of fatty acid under reduced pressure in a stream of carbon dioxide with zinc dust as catalyst and obtained good yields of glycerides. Their procedure was to heat to

200° C. at about 150 mm. pressure for 7 hours then for 3 hours at 240° C. and 120 mm. pressure. (See also Baer and Fischer, 1939.)

Synthesis of fats by enzymes has been successfully demonstrated with castor bean lipase by Welter (1911) and Armstrong and Gosney (1914), who gave an excellent demonstration of the reversibility of the synthetic-hydrolytic powers of the castor bean lipase. Synthesis by the lipase of the pancreatic juice has been demonstrated by Pottevin (1903), Hamsik (1909), Foá (1915), and Artom and Réale (1936) (see also Willstätter and Waldschmidt-Leitz, 1923).

Waxes

Waxes, the esters of the fatty acids with alcohols other than glycerol, are widely distributed in the plant and animal kingdom and, because of their chemical inertness, are useful principally as protective agents. The high lipid content of the tubercle bacillus is due largely to the waxes and wax-alcohols it contains. Insect-waxes and leaf-waxes are mainly esters of higher alcohols and higher saturated acids; thus the chief constituent of beeswax is myricyl palmitate.

The waxes which are of particular importance to the biochemist are the esters of cholesterol and related alcohols with various fatty acids. In animals, these occur in the largest amount in blood plasma, but are present normally in small amounts in most tissues. No mention has been made of such esters in plants. The cholesterol esters of blood plasma have been found to consist mainly of esters of palmitic, oleic, and linoleic acids, with smaller amounts of stearic and other acids. Except for the blood plasma, the esters are to be found normally in notable amounts only in the suprarenal glands. Page and Rudy (1930) have summarized the extensive literature on the occurrence of cholesterol esters in tissues. Small amounts occur in the liver, kidney, heart, and probably in other organs and tissues, but the quantities are generally so small that there is a question whether they are real constituents of the tissues or are due to the blood plasma present. In some abnormal conditions, such as amyloid kidney and in sclerotic arteries, undoubted deposits of esters occur. The fatty livers which occur in depancreatized dogs and in animals fed cholesterol contain considerable amounts of cholesterol esters (Best and Ridout, 1935; Best, Channon and Ridout, 1934; Okey, 1933). The preparation of cholesterol esters from the blood has been described by Hürthle (1896), Hepner (1898), and E. W. Brown (1899).

The waxes are considerably more difficult to hydrolyze than the fats, and this difference has been made use of in an attempt to separate them from the fats (Thaysen, 1914a). For complete saponification of the cholesterol esters, special means must be employed, for instance, long-

continued heating, or the use of sodium ethylate in ethereal solution (Gardner and Fox, 1924). Hydrolysis of the esters by alkali frequently results in a change in the cholesterol itself which interferes with its determination, especially by the digitonin precipitation method. This possibility was first pointed out by Dam (1928), and his findings have been abundantly confirmed since. The separation of cholesterol esters from fats by differential hydrolysis with alkalies has not been found satisfactory with blood, and a separation by the use of enzymes has been worked out by Kelsey (1939b) in this laboratory. Castor bean lipase was found to be inactive toward cholesterol esters but very active toward fat.

Compound Lipids

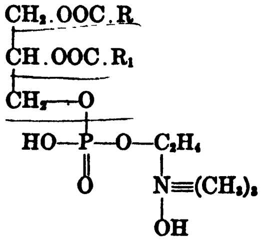
Phospholipids

We are indebted largely to MacLean in England and to Levene and his associates in the United States for clearing up this previously very complicated field and reducing the large number of compounds, mostly poorly defined and doubtful, to a few which are fairly definite. These better characterized members of the group are the lecithins, cephalins, sphingomyelins, and the more recently added plant phospholipids, the phosphatidic acids. These substances contain similar elementary constituents. The lecithins, cephalins, and phosphatidic acids contain two molecules of fatty acid, one of phosphoric acid, and one of glycerol. Lecithin and cephalin contain also an organic base; phosphatidic acid does not. The base is the determining constituent between lecithin and cephalin, although the arrangement of the components may also vary. In lecithin, the base is choline; in cephalin it is aminoethyl alcohol (colamine). Phosphatidic acid is generally found combined with calcium or magnesium which apparently replace the organic base. The sphingomyelins contain one molecule of fatty acid, one of phosphoric acid, two of base, and no glycerol. The bases are sphingosine and choline. The number of phospholipids is limited theoretically only by the number of fatty acids, since each new fatty acid would mean a new compound. Practically, however, the number of different substances of this nature found in the animal body is apparently limited to a few. Evidence is accumulating which indicates that the phospholipid fatty acids vary with those of the food. The phospholipid of cellular material is to be regarded as essential to the life of the cell since it is conserved even in extreme emaciation (Rubow, 1905; Mayer and Schaeffer, 1913); its function is still almost unknown, although it is undoubtedly connected with the metabolism of the fatty acids.

Lecithin. *Structure and composition.* The lecithins and cephalins

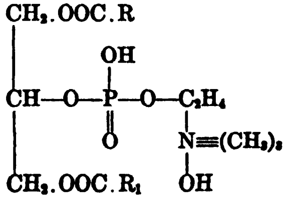
are found associated with each other in all tissues. Whether, as generally assumed, their chemical structure is the same apparently remains to be proved. Thierfelder and Klenk (1930) express the opinion that lecithin is a β-glycerophosphate, and that cephalin is the α form. At any rate, all workers are agreed that it is much more difficult to free the cephalin fatty acids from phosphoric acid and nitrogen than is the case with lecithin.

The generally accepted structure of lecithin is:



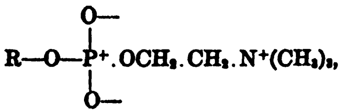
α-Lecithin

This is the asymmetrical or α form. A symmetrical or β form is possible in which the phosphoric acid radical is attached to the middle carbon of glycerol. Suzuki and Yokoyama (1930), and Yokoyama and Suzuki (1931; 1932) have separated the α and β forms of the lecithins from soybean and from human brain on the basis of their differential solubility in acetone. The structural formula for the β-lecithins is:



β-Lecithin

R and R_1 represent various fatty acids. The mode of attachment of the choline to the phosphoric acid is still a matter of dispute, although the ester form of combination shown above is generally accepted. Grün and Limpacher (1923) believe that lecithin exists in the form of an anhydride internally neutralized by the loss of water between the phosphoric acid and choline residues. However, the belief that it occurs in the zwitterion form,



is gaining ground (Jukes, 1934), since it explains a number of points in the behavior of lecithin (Kuhn, Hausser and Brydówna, 1935).

The differences between various lecithins are largely, if not altogether, due to the fatty acids which they contain, and these have not as yet been sufficiently studied. Much of the present information goes to show that each lecithin contains one saturated and one unsaturated fatty acid; but work by Bloor (1928b) and by Sinclair (1932) indicates that this statement may be incorrect, since the solid fatty acid percentage of the total fatty acids found was considerably too low (25-30%), although the liquid (unsaturated) fatty acid percentage was right (about 50%). From this it seems likely that there are some phospholipids which have no saturated fatty acids in their molecules. Levene and Simms (1922) found that the liver lecithins contained the saturated acids, palmitic and stearic, and the unsaturated acids, oleic and arachidonic, although linoleic acid was not excluded. Sueyoshi and Furukubo (1931) found that the main saturated acid of egg lecithin was isopalmitic, and that the unsaturated acids consisted of oleic, clupanodonic, and linoleic acids. Levene and Rolf (1922a) found that egg yolk lecithin contained oleic and small amounts of linoleic and arachidonic acids. In a lecithin prepared from the soybean, Levene and Rolf (1924b) found stearic and palmitic acids, and oleic, linoleic, and linolenic acids. The proportion of unsaturated acids was relatively low as compared with animal lecithins. The fatty acid composition of the animal lecithins may be altered by the fat of the food (Sinclair, 1930).

Anderson (1927b), in his study of the lipids of human tubercle bacilli, described a lecithin which is quite atypical. The only nitrogen present was as ammonia. The molecule contained considerable glucose, a sugar acid, and some glycerophosphoric acid. From the phosphatide of human tubercle bacilli, he isolated inactive inositol and mannose (Anderson, 1930; Anderson and Renfrew, 1930). The fatty acids found were palmitic, a saturated cyclic liquid acid (phthioic), and oleic acid, in amounts in the order named. In avian tubercle bacilli, an optically inactive acid analogous to phthioic acid was found.

Preparation. In preparing phospholipids, advantage is taken of their solubilities. Thus lecithin and cephalin are separated from the other lipids by reason of their insolubility in acetone. Lecithin is separated from cephalin by taking advantage of the insolubility of the latter and its salts in alcohol. They are separated from water-soluble impurities by precipitating their water suspensions with acetone, from organic impurities by combination with metals, *e.g.*, cadmium chloride, and precipitation. The material is first dehydrated, preferably by either alcohol or acetone, keeping in mind the fact that both of these liquids dissolve some phos-

pholipid, especially if there is other lipid present. The phospholipid is then extracted from the dried material by ether and warm alcohol.

The lecithin is purified in various ways. Levene and Rolf (1927a) make use of cadmium chloride followed by ammonia in methyl alcohol, and final emulsification of the lecithin in acetic acid solution. Sueyoshi (1931) treats the extraction residue with acetone, after evaporating off the alcohol and ether, to get rid of undesired substances, especially neutral fat. The material is dissolved or suspended in warm alcohol and set in the cold. Cephalin separates out. The alcohol is evaporated off and the lecithin purified by repeated recrystallizations from acetone. Pure lecithin must have no α-amino nitrogen; the phosphorus-to-nitrogen ratio must be 1:1.

Synthesis. Lecithin has been synthesized by several workers: Grün and Kade (1912), Grün and Limpacher (1923), and by Levene and Rolf (1924a). Levene, Rolf, and Simms (1924) removed one of the fatty acids (the unsaturated acid) by the action of cobra venom on lecithins, and from the lysolecithins so formed built up other lecithins. According to Grün and his associates, the synthesized compounds behave essentially like the natural ones.

Hydrolysis. Lecithin and cephalin are hydrolyzed by the intestinal lipases, setting free the fatty acids, and by a variety of lecithinases which split them in different ways (King, 1934) (see lipases).

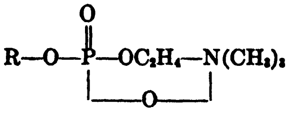
Properties. The properties of the lecithins and cephalins are much the same. They are waxy substances, soluble in the ordinary fat solvents with the exception of acetone, in which they are characteristically insoluble, and alcohol, in which cephalin is insoluble. They are hygroscopic and miscible with water, forming cloudy colloidal solutions from which they may be precipitated by acetone. When pure, they are white in color, but as ordinarily obtained are yellowish. They oxidize readily in air, turning brown and developing a disagreeable odor. They have no definite melting point, but decompose on heating. They form more or less stable combinations with the most diverse substances: salts, proteins, and carbohydrates; and it is probable that they exist in tissues in this type of combination. In plants, they seem to occur mainly in loose combination with carbohydrates (Magistris and Schäfer, 1929). Phospholipids containing, or in combination with, carbohydrates have been found by Anderson (1927b) in bacteria. Both lecithin and cephalin appear to undergo hydrolysis and oxidation more readily than the fats. Their diffusibility through animal membranes has been examined by Süllmann and Verzár (1934), who found that about 25 per cent of the lipids of lipemic blood will pass through membranes of a permeability similar to that of blood capillaries.

The behavior of lecithin with water has been studied by Leathes (1925).

When water comes into contact with a lecithin surface, fingerlike outgrowths of lecithin (myelin forms) into the water take place. Calcium ions inhibit these formations except when cholesterol is present. In films on water, lecithin occupies much more than the area occupied by the fatty acids which it contains, the other groups in the molecule separating the chains and preventing close packing. Cholesterol tends to reduce the surface occupied by the lecithin.

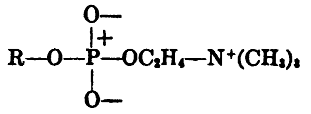
The fact that it is difficult to extract phospholipid from protein solutions or suspensions has led to the belief in a chemical combination between these substances. Lecithoproteins, *e.g.*, vitellin in egg yolk, have been described and for a long time accepted as chemical entities. The present tendency is to regard such compounds as adsorption compounds. De Jong and Westerkamp (1931) studied mixtures of lecithin and various proteins and found that they did form combinations but only within the range of their isoelectric points, that is when they had opposite electrical charges. There appeared then to be no true chemical union. Chargaff (1938) has prepared and described compounds of cephalin and the protein salmine and discussed their possible significance.

Unlike cephalin, lecithin cannot be titrated with a strong base. The reason for this behavior, according to Jukes (1934), is that lecithin is internally neutralized, the strongly acid residual hydrogen of phosphoric acid uniting with the strongly basic choline. It has no buffering power in the physiological range and should probably be written:



Internal anhydride

or better



Zwitterion

Cephalin. The cephalins have been much less studied than the lecithins. They are distinguished from them by their insolubility in alcohol, their content of aminoethyl alcohol (colamine) in place of choline, and possibly by a difference in molecular structure. Cephalin may be titrated as a monobasic acid with a strong base, using phenolphthalein as indicator (Rudy and Page, 1930); it adds one equivalent of base per molecule. Otherwise the properties and behavior of the cephalins are much the same as those of the lecithins (see p. 15).

As the result of titration studies with $N/50$ acid and alkali in benzene containing a little alcohol, Fishgold and Chain (1935) give a somewhat different picture of the ampholytic nature of the phospholipids. They find that lecithin, lysolecithin, cephalin, and sphingomyelin can combine in acid solution with one equivalent of H^+ ions. At alkaline reaction, the phospholipid which possesses a primary amino group gives up one equiva-

lent of H^+ ions. The quaternary bases cannot do so. They conclude that phospholipid with quaternary bases can exist only as cations or zwitterions while cephalin can exist as zwitterions, neutral molecules, anions, or cations.

Composition. Like the lecithins, the cephalins are stated to contain one saturated and one unsaturated acid in each simple molecule, and of these, the saturated acid is apparently stearic. The unsaturated acids of brain cephalin were found by Levene and Rolf (1922b) to be oleic and arachidonic. MacArthur and Burton (1916) found that cephalin from sheep and beef brain contained fatty acid in the following approximate proportions: stearic, 30 per cent; oleic, 55 per cent; cephalinic, 10 per cent; and clupanodonic, 5 per cent. However, as MacLean has pointed out, no one else has found so high a percentage of oleic acid in cephalin. Parnas (1913) came to the conclusion that the only saturated acid of cephalin was stearic. Levene and his associates have spent much time in an attempt to arrive at its composition but have not added a great deal to what Thudichum (1901) had found. Their purest compounds yield only 60-64 per cent of fatty acid as compared with 70-75 per cent for the accepted formula, and the other analytical results do not agree with any known formula. Page and Rudy alone (1932) have described a cephalin (from human brain) with a 75 per cent fatty acid content. A cephalin containing serine has been prepared by Folch (1941).

Preparation. Cephalin may be prepared by the method of Levene and Rolf (1927b). The extracted lipid material dissolved in ether is allowed to stand in the cold, causing the separation of sphingomyelin and the cerebrosides. The cephalin is then precipitated by alcohol to free it from lecithin. The precipitate is dispersed in water and treated with normal hydrochloric acid until a cheeselike precipitate is obtained. It is then separated by centrifugation, washed with acetone, and dried *in vacuo*. It is a powdery, colorless material which quickly darkens in room air. It is soluble in the usual fat solvents except in alcohol and acetone, which precipitate it.

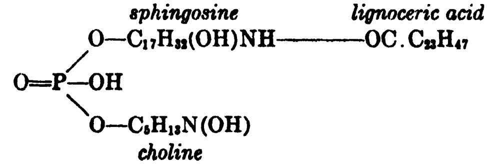
Lysolecithin and lysocephalin. It has been known for a long time that cobra venom acts on lecithin and cephalin to form compounds which are hemolytic. The nature of this action was found to be a partial hydrolysis, and the hemolytic products, half lecithins and cephalins, were isolated by Levene and Rolf (1924a). In both, the unsaturated acid was the one split off, leaving in lysocephalin only stearic, and in lysolecithin, stearic and palmitic acids.

Belfanti (1925) found that lysolecithin from brain or egg yolk dissolves red and white blood cells and injures the brain, producing edema and hemorrhage. Lysolecithins from other tissues are less active, being

readily held in check by cholesterol. King and Dolan (1933) have prepared lysolecithin and lysocephalin by the action of rattlesnake venom on egg-yolk phospholipid. They found them markedly hemolytic. They are hydrolyzed by intestinal mucosa extracts faster than the intact phospholipid and are attacked to a considerable extent by bone phosphatase, which does not act on the intact phospholipids. Chargaff and Cohen (1939) prepared lysophospholipid from egg yolk and free lecithin and cephalin, using the venoms of the moccasin, cobra, and fer-de-lance. They found that in contrast to the thromboplastic effect of cephalin, lysocephalin lacked any influence on the blood-clotting mechanism.

Sphingomyelin. Sphingomyelin occurs in brain, kidney, liver, egg yolk, and, in small amounts, in blood and muscle. Together with cerebrosides, it constitutes the substance called protagon.

Composition and structure. The substance as prepared by Levene (1916) yielded two fatty acid radicals, one probably lignoceric, and about an equal amount of a low-melting acid, probably a hydroxy acid; two bases, sphingosine and neurine or choline; and phosphoric acid. According to Levene, sphingomyelin contains no unsaturated acid. The formula suggested by him is as follows:



Merz (1930), while confirming Levene's formula, found stearic, lignoceric, and nervonic acids in sphingomyelin and believed that there are at least three sphingomyelins, differing from one another only by their fatty acid component.

Sphingosine, the chief base, is an unsaturated amino-alcohol containing two hydroxyl groups, one primary amino group, and one double bond, with the empirical formula, $C_{17}H_{35}NO_2$, and with the probable composition, $CH_3(CH_2)_{11}CH{=}CH.CHOH.CHOH.CH_2NH_2$.

Properties. The known sphingomyelins are relatively stable substances, undergoing no change in air or light. They are soluble in hot alcohol or pyridine, from which they separate on cooling in crystalline form, relatively insoluble in cold or hot ether, easily soluble in cold or hot chloroform, benzene, and glacial acetic acid. They are insoluble in cold, but somewhat soluble in hot, acetone. They mix with water to form a relatively permanent opalescent suspension, from which they are precipitated by acetone. They are dextrorotatory, having a specific rotation of about 8.

Preparation. According to Levene (1914) sphingomyelin may be prepared as follows: Dried brain is exhaustively extracted with boiling alcohol, the extract chilled, and the precipitate thoroughly extracted with ether and acetone. The residue is dissolved in hot pyridine, the extract cooled, filtered, and the precipitate dissolved in hot glacial acetic acid. A precipitate separates on cooling and is filtered off. The acetic acid containing the sphingomyelin is concentrated in vacuum and the sphingomyelin precipitated by acetone. The raw product is further purified (Levene, 1916) by solution in a mixture of five parts of petroleum ether and one part of alcohol, and alcohol is added as long as precipitation occurs. After standing overnight at 0° and filtering, the extract is concentrated and poured into acetone. The precipitate is purified further by several recrystallizations from a mixture of equal parts of chloroform and pyridine until free from cerebrosides. The pure substance has a P:N ratio of 1:2 and gives no test for carbohydrate (cerebrosides) or free amino nitrogen (Levene, 1914).

Phosphatidic acids. Chibnall and Channon (1929) have prepared from various plant sources (*e.g.*, leaves of cabbage) compounds which differ from lecithin and cephalin in that the base, choline or aminoethyl alcohol, is replaced by calcium. From these compounds the corresponding diglyceride phosphoric acids, phosphatidic acids, were prepared and described. They are thick oils which oxidize readily, becoming dark and insoluble in ether. Their properties are very similar to those of the other phospholipids; consequently, the methods of isolation are very similar to those used for lecithin and cephalin. The fatty acids present are palmitic and stearic, linoleic and linolenic, but no arachidonic. The unsaturated acids greatly exceed the saturated in amount so that, as is the case in the phospholipids of the animal body, there must be phosphatidic acids containing only unsaturated acids. The phosphatidic acids are apparently confined to plant tissues, although older work quoted by Chibnall and Channon indicates that if not actually present in animal tissues, they may be formed from the ordinary phospholipids by the processes used in separation or by post mortem changes (Levene and Komatsu, 1919). Work by Jordan and Chibnall (1933) indicates that these compounds, like the ordinary phospholipids, are integral parts of the plant protoplasm and perhaps replace phospholipids in cellular metabolism.

Glycolipids (galactolipids or cerebrosides)

The glycolipids are those compounds in which there are present a sugar, a fatty acid, and an organic base, but no phosphoric acid. The only ones known are the compounds containing galactose. These are better known as the cerebrosides, of which four, phrenosin or cerebron, nervone, hy-

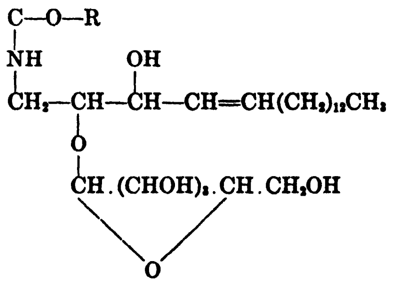
droxynervone, and cerasin (Klenk, 1928), are fairly well characterized. They differ from one another only in the nature of the fatty acid which they contain: phrenosin containing cerebronic or phrenosinic acid ($C_{24}H_{48}O_3$); nervone, nervonic acid ($C_{24}H_{46}O_2$); oxynervone, oxynervonic acid ($C_{24}H_{44}O_3$); and cerasin, lignoceric acid ($C_{24}H_{48}O_2$). They are white amorphous solids.

In solubility, the cerebrosides closely resemble sphingomyelin, and their separation from this substance is difficult. Together with it, they were the chief constituents of the protagon which filled so many pages of the literature a generation ago. They dissolve readily in pyridine, hot alcohol, acetone, or benzene, but are almost insoluble in ether, hot or cold.

The cerebrosides occur in largest amounts in the brain and nerves. In the brain, they appear to be confined to the white matter. They form an important constituent of the myelin sheath of nerves and appear to be developed along with it after birth. They are found, in small amounts, in other normal tissues and accumulate, in large amounts, in the spleen and liver in Gaucher's disease.

They are of special interest to the biochemist because they contain galactose, which is not known to occur in any other combination in the tissues; also because they contain a sugar and a fatty acid in the same molecule, which is of theoretical importance from the fact that the fatty acids in the later stages of their metabolism seem to require the assistance of carbohydrate for combustion. The significance of these compounds in the living body is not known, and their mention in the biochemical literature is mostly in connection with their separation and characterization.

The formula usually given for the cerebrosides is as follows:



Fatty acid

Sphingosine

Galactose

Sulfolipids

Lipids containing sulfur have been described by Koch (1902; Koch and Koch, 1917), and one was obtained by Levene and Landsteiner (1927) from the white matter of horse kidney. Koch gave the following figures for the lipid sulfur of tissues in percentage of dry substance: for muscle,

0.008 per cent; salivary gland, 0.018; testicles, 0.023; liver, 0.026; gray matter of brain, 0.03; white matter, 0.180. Sulfolipids are also reported in tumors by Bolaffi (1931). Blix (1933) identified the lipid sulfur compound of brain as cerebron sulfuric acid ester, and found that about one-fifth to one-quarter of the brain cerebroside is so combined.

Derived Lipids

Fatty Acids

Occurrence

Saturated acids. *Straight-chain* ($C_nH_{2n}O_2$). Most of the even-numbered carbon atom members of the saturated straight-chain or acetic acid series are to be found in plant and animal bodies combined both in neutral fat and in the more complex lipids. It is a notable fact that odd-numbered carbon acids do not occur in either plants or animals in significant amounts, although it has been demonstrated that they can be used by animals. The fatty acids occurring naturally in plants and animals are almost always of the normal straight-chain form.

In Table 1 is a list of the commonly occurring fatty acids with some of their characteristics.

Saturated cyclic acids. Phthioic acid, which is isomeric with cerotic acid, and occurs in tubercle bacilli, was described by Anderson (1929). It is apparently the cause of some of the clinical manifestations of the disease (proliferation of the epithelioid cells and epithelioid giant cells). It is a liquid saturated acid, m.p. 28°C.; optical activity $[\alpha]_D = 7.98$; molecular weight 396. *Tuberculostearic acid* ($C_{18}H_{36}O_2$), which is similar to phthioic acid, is an important constituent of the lipids found in these bacteria, but is optically inactive and has no biological activity comparable to phthioic acid.

Unsaturated acids. Members of all the series containing from one to five and possibly six double bonds are found in plant and animal fats. Isomers of different types occur, and since the proportion of the different isomers seems to change with treatment, with resulting change of properties, the difficulty of separation and of identification of the members of the different series is very great; for example, elaidic acid (an isomer of oleic acid) is solid at ordinary temperatures, and during the separation of solid and liquid acids by the lead salt-alcohol process it appears in considerable amounts among the solid acids (Sinclair, 1935). Brown (1929a) found that the ether-insoluble polybromide of arachidonic acid represented only about one-quarter of the total acid, the polybromides of the other isomers being soluble in ether. He suspected also that the proportion of the isomers changed with treatment. Other difficulties in the examination of the

Table 1. Distribution of Fatty Acids.

C Atoms	Double Bonds	Name: Common	Name: Chemical	Where Found: Plant Kingdom	Where Found: Animal Kingdom
4	0	Butyric	Butanoic	Bacteria	Butter fat
6	0	Caproic	Hexanoic	Coconut, palm kernel oil	Butter fat
8	0	Caprylic	Octanoic	Coconut, palm kernel oil	Butter fat
10	0	Capric	Decanoic	Coconut, palm kernel oil	Butter fat
	1		9-Decenoic		Butter fat
12	0	Lauric	Dodecanoic	Coconut, laurel oil, etc.	Not found in butter fat
	1		Dodecenoic	Lindera obstiroba	Butter fat
14	0	Myristic	Tetradecanoic	Coconut oil, etc.	Butter fat, rat and hen body fats, fish oils, frog fat
	1	Myristoleic	Tetradecenoic		Butter fat, sperm oil, fish liver oils
16	0	Palmitic	Hexadecanoic	Most plant fats	Most, if not all, animal fats
	1	Palmitoleic	Hexadecenoic	Lycopodium spores, yeast phospholipid	Butter, hen and rat fats, frog fat
18	0	Stearic	Octadecanoic	Plant fats in general	Most animal fats; present in small amounts only in fish liver oils
	1	Oleic	9-Octadecenoic	Present in most, if not all,	plant and animal fats
	2	Linoleic	9,12-Octadecadienoic	Many plant fats	Butter (?), most mammal fats in greater or less amount, fish liver oils, frog fat, insects
	3	Linolenic	9,12,15-Octadecatrienoic	Many plant fats	Butter (?), small amounts in mammalian fats, fish liver oils, frog fat, insects
	4	Stearidonic	Octadecatetrenoic		Cod liver oil
20	0	Arachidic	Eicosanic	Peanut, rape oil, etc.	Butter fat, lard
	1	Gadoleic	9-Eicosenic		Human brain phospholipids, fish liver oils
	4	Arachidonic	Eicosatetraenic	No recorded occurrence in plant fats	Animal phospholipids, butter fat, liver fats of mammals, fish and frog
22	0	Behenic	Docosanoic	Oil of ben, etc	Butter fat (?)
	1	Erucic	13-Docosenoic	Rape oil, etc.	Isomer in sardine oil
	5	Clupanodonic	Docosapentenoic	No record	Brain phospholipids, liver phospholipids, fish liver
24	0	Lignoceric	Tetracosanoic	Peanut oil, etc.	Animal cerebrosides and sphingomyelin
	1	Nervonic	Tetracosenoic	No record	Cerebrosides

unsaturated acids are caused by their relative ease of oxidation, especially in alkaline solution, and by the fact that they polymerize readily. A practical difficulty is that they and their soaps readily form emulsions with water and solvents, which are difficult to break.

The number of isomers of the more highly unsaturated acids may be great. For example, Brown (1929a) has calculated that the number of possible isomers of arachidonic acid is 256. Actually the number occurring in plant and animal tissues is small. One limitation on the number is illustrated by an unexplained regularity, noted in a review of the unsaturated acids by André (1925). André's observation was that in by far the greater number of the unsaturated acids no double bond was found between the end carbon atom (generally the carboxyl carbon) and the carbon atom nine places away; that is, in all the naturally occurring unsaturated fatty acids a nine-carbon portion of the chain is free from double

bonds. He also pointed out another regularity which apparently further limits the number of naturally occurring isomeric unsaturated fatty acids, namely, that it is rare to find double bonds on adjacent carbon atoms. According to the rule, there must be a saturated carbon between pairs of double-bond carbons. For this reason, it would not be possible to have more than three double bonds in an eighteen-carbon chain, which seems to be the case; the best known four-bond acid which occurs in significant amounts in animal tissues is arachidonic acid, which is a C_{20} acid. Acids of a different make-up are, however, occasionally reported. Thus, Van Loon and Steger (1931) describe a fatty acid (from *Couepia grandiflora*) which they call couepic, a C_{18} acid with three double bonds on adjacent carbons. Tung oil contains, in large amount, eleostearic acid ($C_{18}H_{32}O_2$) containing three conjugated double bonds. Fatty acids with double bonds between the ninth carbon and the carboxyl group are not known to occur in mammals.

Oleic series ($C_nH_{2n-2}O_2$). Of the oleic series, only oleic acid is found in large amounts, and the one with the double bond in the 9-10 position is by far the most common. Another with the double bond in the 12-13 position has been described by Hartley (1907; 1909) but denied by later workers (Channon, Irving and Smith, 1934).

Oleic acid may be changed to its isomer, elaidic acid, by treatment with nitrous acid or by heating with sulfuric or phosphoric acids. This substance has a melting point of 44°C. and behaves like a solid acid in the lead salt-alcohol separation. It is partly responsible for the iodine absorption of the solid fraction of the fatty acids. As in oleic acid, the double bond is in the 9-10 position, but Bertram (1928) has described a 10-11 elaidic acid.

Other acids of relatively rare occurrence are tiglic (C_5) in croton oil, several C_{16} acids, gadoleic (C_{20}) in cod liver oil and other fish oils, erucic (C_{22}), with double bond between the thirteenth and fourteenth carbons, and its isomers in vegetable oils, and nervonic ($C_{24}H_{46}O_2$) in the cerebrosides. Rapic acid ($C_{18}H_{34}O_2$) in rape and colza oil is unusual in that it does not form a solid modification with nitrous acid. Klenk (1927) determined the position of the double bond in nervonic acid by the ozonide method. It was found to be in the regular 9-10 position from the carboxyl group, the remainder of the molecule being $=CH(CH_2)_{13}CH_3$ with an unbranched chain. On hydrogenation it yielded lignoceric acid.

Linoleic or linolic series ($C_nH_{2n-4}O_2$). The more common acids of this series are all C_{18} acids. As the name indicates, they were found first in linseed oil. The double bonds are in the position expected from André's rule (1925), *i.e.*, 9-10 and 12-13. Hartley (1907; 1909) has pre-

pared a linoleic acid from pig's liver which is probably the same as that found in linseed oil.

Linolenic series ($C_nH_{2n-6}O_2$). Acids of this series occur widely in plants but are not commonly found in animal tissues. Linolenic acid may be prepared from linseed oil. Certain irregularities show up in the properties of this acid as pointed out by Knauss and Smull (1927). The iodine number was found to be 223-227 instead of the theoretical 274. The rate of bromination is slower than with less unsaturated acids. These irregularities are thought to be due to isomeric transformations. The fact was noted that the acid, regenerated from the hexabromide and then rebrominated, gave only about one-quarter of the original insoluble hexabromide, indicating a change in the molecule resulting in isomers which did not form insoluble bromides (Erdmann and Bedford, 1909). Decomposition of the mixture of α and β linolenic acids gave azelaic acid and azelaic acid semialdehyde, the latter in larger quantity (Erdmann, Bedford and Raspe, 1909); these compounds indicate that there was a nine-carbon portion of the molecule free from double bonds.

Arachidonic series ($C_nH_{2n-8}O_2$). Since the acids of the arachidonic series have four double bonds, in accordance with André's rule they should have at least twenty carbon atoms. The only one which is at all widely distributed is arachidonic acid ($C_{20}H_{32}O_2$). It is found in practically all animal tissues, both in the phospholipids, in which it is present in relatively large proportions, and in the stored fats. Wesson (1925) found 0.68 to 0.75 mg. per gram of tissue in the whole animal (rat), a value which could be raised to 2 or 3 mg. per gram of tissue by feeding cod liver oil. Fasting resulted in slightly larger values. Pig's liver contained 1.3 to 1.5 grams per kilo; dog's liver, 1.8 grams; pancreas, 1 gram; kidney, 1.1 grams; heart muscle, 0.1 gram. In general, Wesson found that increase of fat metabolism meant increase of arachidonic acid. Brown and Ault (1930) found arachidonic acid in beef, hog, and sheep brains, and in sheep and beef brains there was probably a still more unsaturated acid: tetracosapentenoic ($C_{24}H_{38}O_2$). In lard, Brown and Deck (1930) found from 0.3 to 0.4 per cent of arachidonic acid. In liver, Brown (1928) found arachidonic to be the only unsaturated acid present and it occurred in amounts from 2.0 to 7.7 per cent of the total fatty acids. Klenk (1929) found no C_{20} acid in brain, only C_{18} and C_{24} acids; but in later work (1931) he reported that the C_{20} unsaturated acids were present, possibly as oxidation products of the C_{24} acids.

Clupanodonic acid ($C_{22}H_{34}O_2$) was found by Tsujimoto (1921) in various fish oils and reported by Sueyoshi and Furukubo (1931) in egg lecithin, in which the ether-insoluble bromides corresponded to a C_{22} double-bond

acid. The composition of the unsaturated acids of egg lecithin was: oleic, 73 per cent; clupanodonic, 5 per cent; linoleic, 2 per cent.

Unsaturated hydroxy acids. *Ricinoleic* acid ($C_{18}H_{34}O_3$) occurs mainly in castor oil, of which it is the characteristic acid. *Oxynervonic* acid ($C_{24}H_{46}O_3$), found in the cerebrosides, apparently belongs here.

Unsaturated cyclic acids. *Chaulmoogric* ($C_{18}H_{32}O_2$) and *hydnocarpic* ($C_{16}H_{28}O_2$) acids occur in chaulmoogra oil and are of interest because of their therapeutic value in the treatment of leprosy. They are irritating to the intestine, increasing peristalsis. Intravenously, they cause hemolysis.

Properties of the fatty acids.

Solubility. The lowest members of the saturated fatty acid series up to C_6 are miscible with water in all proportions. Caproic acid is soluble in water at 15°C. to the extent of about 0.9 per cent, and the solubility of the series decreases rapidly with increasing length of chain. All the saturated acids above lauric acid are practically insoluble in water. The alkali salts of palmitic acid are soluble in water, but its other metallic salts are generally insoluble in water and often in other solvents, such as alcohol. The lead salt, for example, is insoluble in alcohol, and this fact is made use of in separating it from the unsaturated acids.

In hot absolute or 95 per cent alcohol, most of the fatty acids are soluble, but from palmitic acid upward all are sparingly soluble in cold alcohol. All fatty acids are soluble in ether, chloroform, benzene, etc. All except the hydroxy fatty acids are soluble in petroleum ether (distilling below 60°C.).

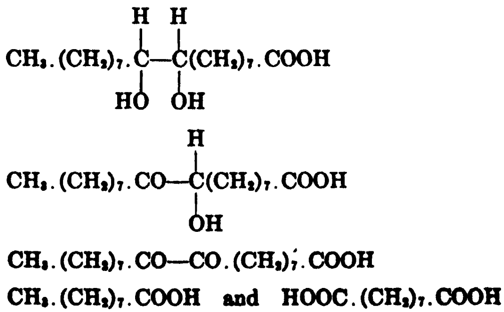
The solubility of the hydroxy acids in water depends upon the number of hydroxyl groups; thus octahydroxyarachidic acid is easily soluble in water (Lewkowitsch, 1921), hexahydroxystearic more difficultly soluble, and tetrahydroxystearic acid requires 2000 parts of boiling water for solution. The highly hydroxylated acids are insoluble in ether and difficultly soluble in alcohol. The dicarboxy acids are more soluble in water than the corresponding monocarboxy acids and, in general, less soluble in the fat solvents. The solubilities of the hydroxy and dicarboxylated acids are of potential importance in biochemistry, since all reactions of living beings take place in a watery medium and probably in water solution. The fact that the fats are insoluble in water imposes a difficulty on their utilization and gives importance to any compounds which are soluble in or miscible with water. The dicarboxy acids are among the products of oxidation *in vitro* of the unsaturated acids, but their presence in the tissues of animals in significant amounts has not been demonstrated, except possibly in the case of some of the simpler ones as, *e.g.*, succinic (see below).

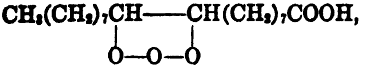
Dicarboxylic acids appear in the urine in small amounts during the metabolism of the fatty acids of intermediate length of carbon chain (maximum with C_9) (Verkade and van der Lee, 1934).

Salts of glycocholic and taurocholic acids (bile salts) dissolve fatty acids and greatly increase the solubility of the soaps formed during digestion of the fats (Moore and Parker, 1901; Verzár and Kúthy, 1929).

Melting point. The melting points of the saturated fatty acids give a steplike curve with increase of carbon content, the next higher odd-numbered acid having a lower melting point than the even-numbered acid with one less carbon atom. Both odd and even members of the series by themselves give smooth curves. The acids of the series up to and including the acid containing 10 carbons are liquids at ordinary temperatures. The naturally occurring unsaturated acids of all series are liquid at ordinary temperatures, and it has been noted that in some respects they behave like acids of a much shorter chain length. For example, oleic acid behaves, as regards melting point (14°C.), like an acid of approximately half its chain length. On the other hand, its isomer, elaidic acid, has a melting point (45°C.) intermediate between that of oleic acid and the corresponding saturated stearic acid. The double bond of itself need not greatly change the melting point as the following values show: stearic acid 69°C., oleic acid (Δ 9-10) 14°C., linoleic acid 18°C., oleic (Δ 2-3) 59°C., oleic (Δ3-4) 56°C., oleic (Δ 4-5) 52°C., oleic (Δ 6-7) 33°C., elaidic 52°C., and eleostearic 49°C. The melting point of pelargonic acid ($C_9H_{20}O_2$), 12.5°C., is suggestively close to that of ordinary oleic acid (14°C.).

Oxidation. Oxidation of oleic acid *in vitro* yields a variety of products depending on the oxidizing agent and the conditions under which oxidation takes place. With potassium permanganate in alkaline solution at a low temperature, it yields a dihydroxystearic acid, and at a higher temperature, it breaks at the double bond giving two nine-carbon acids: azelaic, a dicarboxy acid; and pelargonic, a monocarboxy acid. The stages in oxidation are probably as follows (Leathes and Raper, 1925):



Ozone, acting on oleic acid, forms first an addition product, an ozonide,  which, on heating with potassium alcoholate yields azelaic and pelargonic acids, thus giving evidence that the double bond is in the middle of the molecule. It is assumed that the position of the double bond does not shift during the oxidation. That the double bond in oleic acid may be mobile and that therefore the foregoing assumption may be incorrect is believed by Armstrong and Hilditch (1925); and in addition there is the well known fact that on fusing oleic acid with potassium hydroxide, it yields palmitic acid, indicating a displacement of the double bond from the 9-10 to the 2-3 position. There is as yet no evidence of a shifting of the double bond during the metabolism of the fatty acids. Whether the double-bond acids form ozonides or similar peroxides in the living body has not been shown; but the indications are that they do, and that these compounds take some part in oxidations (Hopkins, 1925; Meyerhof, 1923).

The fact that the double bond was shown to be a point of weakness in the chain, at least during oxidation *in vitro*, has been taken by Leathes to indicate that during oxidation in the animal body a similar breaking of the chain takes place. Exception may be taken to this assumption, as Leathes himself has pointed out, by reason of the facts that there is no evidence for the formation of either azelaic or pelargonic acids in the body, and that, when fed, azelaic acid is not utilized. Other studies on this phase of oxidation of the fatty acids are those of Skellon (1931) and Ellis (1932). The final stage in the breakdown of the fatty acid chain in the animal body is mainly by a process of β-oxidation with loss of two carbon atoms at a time. The details are discussed in Chapter V (Metabolism).

Hydrogenation. The unsaturated acids take up hydrogen at the double bonds with the aid of catalysts and become saturated. Oleic acid, for example, may be saturated with hydrogen by the use of a metallic catalyst (nickel), yielding the corresponding saturated acid (stearic). Sabatier (1923) and his co-workers were the first to show that finely divided metals, particularly nickel, are the best catalysts. Since that time, the process has become of great commercial importance in changing comparatively undesirable oils, such as cottonseed oil, into desirable articles of diet. As far as can be determined, hydrogenated fats are just as well utilized by the animal body as natural fats (Langworthy and Holmes, 1915). As regards sequence of hydrogenation of the double bonds in the poly-unsaturated acids, there is a difference of opinion. Suzuki and Inoue (1930), for example, found that in the reduction of linolenic acid, the

ethylene linkage farthest from the carboxyl group was saturated first; but Bauer and Ermann (1930) found just the reverse. Supporting Suzuki and Inoue is the work of Dowell (1921) who found, as they did, that the 9-10 linkage is the last to be saturated. On the other hand, Longenecker (1939) found that utilization in fasting produced only slight changes in the depot fat in rats.

Dehydrogenation, the opposite of hydrogenation, is difficult and has not been accomplished commercially, although the reward for such a process would be great, for example, if the drying oil necessary for the paint industry could be made from cheap hard fats such as tallow. In the animal body, the ability to desaturate seems limited; otherwise there would be no need for the addition of "essential" fatty acids, linoleic or linolenic acids, to the diet (see Chapter V).

Halogen absorption. The halogens are readily absorbed at the double bonds, and, under certain conditions, the absorption is quantitative, so that the halogen absorption value (iodine number) constitutes one of the most important means of study of the fatty acids. The more unsaturated acids (three and four double bonds) form halogen absorption derivatives which are insoluble in most fat solvents, and this property has been used for their identification. Some of their peculiarities of behavior toward the halogens have been discussed by Caldwell and Piontkowski (1934). These workers found that proximity to the carboxyl group interfered greatly with the absorption of halogen. In the naturally occurring fatty acids the double bonds are generally so far removed from the carboxyl group that it has no effect on the absorption. It is notable that while the naturally occurring acids with one and two double bonds take up the theoretical amounts of halogen, those with more than two double bonds fall short of the theoretical absorption. Thus, ordinary oleic and linoleic acids have iodine numbers 90 and 181, showing complete saturation of the compounds with halogen; linolenic has an iodine number of 223 (theoretical 274). Conjugation of the double bonds as in eleostearic acid interferes with complete halogen saturation (actual iodine number 169, theoretical 274).

Surface properties, films, etc. Much highly significant work on this topic has been done by Langmuir (1917) and more recently by Adam and associates (1921-1928). For details of this work, which is outside the field of this discussion, the reader is referred to these papers. The fatty acids and their soluble soaps greatly lower the surface tension of water.

Soaps. The fatty acids, like other acids, combine with metals to produce salts, of which those of the longer-chain acids are called soaps. Only the soaps of sodium, potassium, and ammonium are soluble in water, but

the insoluble soaps of some of the other metals, *e.g.*, calcium and magnesium, are also of interest to the biochemist. Since the fats are believed to be completely hydrolyzed in the intestine before absorption, and in the presence of the alkaline secretions of the pancreas and liver (bile) would form soaps, the presence of the latter in the intestine has always been assumed. However, since the intestine generally is on the acid side of the neutral point throughout most of its length, considerable doubt has been expressed regarding the presence of any considerable amount of soaps in the intestine. Moreover, Raper (1913) found that the soluble soaps caused severe destructive changes in the intestinal mucosa and harmful cytolysis when injected into the tissues. When there is excess of calcium or magnesium, there may be considerable loss of fatty acid through the feces due to the formation of insoluble and unabsorbable soaps.

The soaps are good emulsifiers and it has generally been assumed that they act as emulsifiers of the food fat in the early stages of digestion. However, emulsions are not always found (Moore and Rockwood, 1897) even when fat digestion is in progress. Because of the fact that the reaction of most of the intestine is not favorable for soap formation, it is not certain to what extent soap is necessary for fat digestion (see Chapter II).

Much work has been done on the nature and properties of soap solutions (McBain and associates, 1922), but since it has no direct bearing on the biochemistry of the fatty acids, its discussion is out of place here.

The germicidal and detoxifying actions of soap have been studied by several investigators, and there is considerable difference of opinion as to whether or not the soaps have germicidal properties, with the balance of opinion tending more and more to the positive side. Lamar (1911) studied the effect of soaps plus antisera on pneumococcus and found that the more unsaturated the fatty acid (iodine number) the greater its lytic effect. The greatest activity was shown by potassium linoleate. The toxicity of the soaps toward bacteria increases with length of chain (Eggerth, 1931). The detoxifying effect of soaps on bacterial toxins has been investigated by Larson and associates (1925). They made detoxification tests with sodium salts of oleic, stearic, palmitic, myristic, lauric, and ricinoleic acids by mixing equal volumes of standard toxin with 1 per cent soap solutions and injecting into guinea pigs. The ricinoleate was by far the best detoxifying agent; oleate, stearate, and palmitate had no effect at all, and the others only a slight one. The detoxification was evidently an adsorption phenomenon; the soap was adsorbed on the toxin and so inactivated it. The toxin was not destroyed, however, and could be used as an antigen, since it was released slowly over a period of time.

Alcohols in natural combination with the fatty acids

Glycerol [$C_3H_5(OH)_3$]. Glycerol is the commonest of the alcohols found in combination with the fatty acids, and it exists not only in the ordinary fats but in the phospholipids, which are also triglycerides. It is a triatomic alcohol and hence may form mono-, di-, or tri-compounds; only the tri-compounds, however, exist in nature in important amounts. There are two chemical positions in the molecule, the end groups constituting one and the middle group, the other. The end groups are said to be in the α, and the middle group in the β position. Two groups of structural isomers are therefore possible.

The three carbon atoms also make possible optical isomerism if the middle carbon is unequally loaded, as is the case if all three hydroxyls are replaced by different groups, or even if the two α groups are different (see discussion of the fats, p. 8). Optical isomerism is, however, never realized in the natural triglycerides or fats, no fats of the ordinary fatty acids being known which are appreciably optically active. When the fatty acids themselves are optically active, as is the case with the cyclic acids, the glyceride is optically active. In the more complex triglycerides (phospholipids) optical activity is always present, and, since most compounds in nature taking part in life processes are optically active, some significance may be attached to this fact. The fats are to be regarded as merely stores of calories, but the phospholipids are active cellular constituents.

The glycerides, both fats and phospholipids, are fairly easily hydrolyzed, especially by alkali. Hydrolysis takes place much more readily in a solvent, such as alcohol, in which the glycerides and the hydrolyzing agent are both soluble. The products of hydrolysis are glycerol and the alkali salts of the fatty acids (soaps). To separate the glycerol, the hot aqueous solution is acidified, whereupon the fatty acids separate as insoluble material and come to the top of the solution, leaving the glycerol in the water. Glycerol is also obtained by the action of yeast on sugar under special conditions (Neuberg and Färber, 1917; Neuberg and Reinfurth, 1918).

Glycerol is miscible with water in all proportions, is highly hygroscopic, and forms a crystalline solid when cooled, the crystals melting at 20°C. It distills with steam and so cannot be obtained water-free by heating. It dissolves readily in alcohol but only slightly in ether and is practically insoluble in other fat solvents, so that extraction of the acid hydrolysate of fat with fat solvents provides a good means of separation from the fatty acids. Glycerol boils at 290°C., generally with some decomposition, especially when impurities are present, and the decomposition is con-

siderably faster when dehydrating agents such as acid-potassium sulfate are present. Under these conditions, it loses two molecules of water and forms acrolein, the presence of which serves as a test for glycerol. Glycerol esters with other than fatty acids are sometimes of physiological interest, *e.g.*, those with nitric acid, with which it forms nitroglycerin, and with phosphoric acid, with which it forms glycerophosphoric acid. Of the latter acid, both the α and β isomers are found, and this form of isomerism occurs in the phospholipids (Suzuki and Nishimoto, 1930; Karrer and Salomon, 1926; Rae, 1934). For details regarding the chemistry of glycerol the reader is referred to Lawrie (1928).

Straight-chain alcohols. A variety of other alcohols, with straight carbon chains, both saturated and unsaturated, have been found combined with the fatty acids or associated with the lipids. Except as compounds of the fatty acids used by living organisms mainly for structural or protective purposes, they have no known function and therefore call for no discussion. A list of the more common ones is given in Table 2.

Table 2. Alcohols (from H. B. Bull, 1937).

Name	Formula	Occurrence
Lanolyl	$C_{12}H_{24}O$	Wool fat
Cetyl	$C_{16}H_{34}O$	Sperm oil
Octodecyl	$C_{18}H_{38}O$	Sperm oil, rump gland wax
Arachyl	$C_{20}H_{42}O$	Dermoid cysts
Carnaübyl	$C_{24}H_{50}O$	Wool fat
Ceryl	$C_{26}H_{54}O$	Many waxes
Melissyl (myricyl)	$C_{30}H_{62}O$	Waxes
Oleyl	$C_{18}H_{36}O$	Fish liver oil
Chimyl	$C_{19}H_{40}O_3$	Fish liver oil
Sebrachyl	$C_{21}H_{42}O_3$	Fish liver oil
Batyl	$C_{21}H_{44}O_3$	Fats and oils

The sterols. Of the other alcohols, cholesterol and phytosterol are the best known, cholesterol being characteristic of animal tissues, and the phytosterols (a general term including several sterols) being the corresponding alcohols in plants. The distribution of the sterols in various fats and oils has been reported on by Steuart (1923), and by Anderson and Moore (1923). Cholesterol and the phytosterols are related compounds differing from one another in molecular weight, crystal form, melting point, degree of optical rotation, and melting point of esters. Cholesterol and many of the plant sterols have been quite well studied, so that their structure is definitely known (see Strain, 1938). Windaus and Hauth (1906) found in the phytosterol of the calabar bean two sterols, sitosterol and stigmasterol. Sitosterol ($C_{29}H_{50}O$) gives the color reaction of cholesterol. Stigmasterol ($C_{29}H_{48}O$) contains two double bonds and gives the Salkowski and the Liebermann-Burchard reactions. In spite of the similarity in composition, cholesterol and the phytosterols are not interchangeable in the animal body. The most recent work shows that

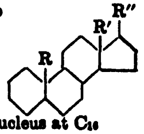
plant sterols are not absorbed from the gastrointestinal tract and are not changed into cholesterol.

An entirely new and fascinating chapter in the chemistry and physiology of the sterols has been opened up by the demonstration of their relationship to the bile acids, vitamin D, the sex hormones (Butenandt, 1936; Adam, Danielli, Haslewood and Marrian, 1932), the cardiac aglucones (Elderfield and Jacobs, 1934), toad poisons (Chen and Chen, 1933), etc., all of which have the characteristic basic structure of the sterols and some of which have been made from the sterols. Table 3, from Strain (1938), illustrates some of these relationships.

Table 3. Members of the Cyclopentanoperhydrophenanthrene Group (Strain, 1938).

Member	Class of Compound	Side Chains Attached to Ring System* R	R'	R''	Sources
Sterols	Saturated and unsaturated secondary alcohols	$-CH_3$	$-CH_3$	CH_3 over $-CH_2$ or $-CH(CH_3)-(CH_2)_3-CH(CH_3)_2$ or substituted isoöctyl with or without unsaturation	Tissues of animals, plants, and fungi
Bile Acids	Hydroxy acids	$-CH_3$	$-CH_3$	$-CH(CH_3)-CH_2-CH_2-CO_2H$ (generally)	Bile
Heart Poisons Cardiac aglucons	Unsaturated hydroxy lactones	$-CH_3$, $-CH_2OH$ (?) or $-CHO$	$-CH_3$	$CH_2-C{=}O$, O, $-C{=}HC$ (five-membered lactone ring) or $CH{=}CH-C{=}O$, $-C{=}CH-O$ (six-membered lactone ring)	Leaves, seeds, and roots of digitalis and related plants
Toad poisons	Unsaturated hydroxy and acetoxy lactones	$-CH_3$ (?)	$-CH_3$	$CH{=}CH-C{=}O$, $-C{=}CH-O$ (?)	Parotid secretion of toads
Digitalis Sapogenins	Hydroxy cyclic ethers	$-CH_3$	$-CH_3$	$-CH(CH_3)-CH-CH-CH_2$† with O to (C_{16}), O and $CH-CH_3$ joined through CH_2	Leaves, seeds, and roots of digitalis, etc.
Sex Hormones Estrogenic	Phenolic alcohols or ketones	......	$-CH_3$	$=O$ or $-OH$	Gonads, placenta, urine
Corpus luteum	Unsaturated diketone	$-CH_3$	$-CH_3$	$-CO-CH_3$	Corpus luteum
Androgenic	Saturated and unsaturated keto-alcohols or dihydric alcohols	$-CH_3$	$-CH_3$	$=O$ or $-OH$	Gonads, urine
Adrenal substances	Unsaturated ketonic alcohols	$-CH_3$	$-CH_3$	$-CH(OH)-CH_2OH$, $-C(=O)-CH_2OH$, etc	Adrenal glands

* Ring system of the steroid group



† A furan ring is formed with the nucleus at C_{16}

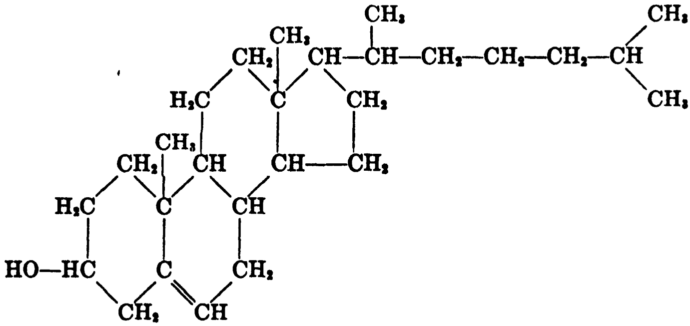
Cholesterol. *Occurrence.* Cholesterol is a characteristic constituent of all animal tissues. In warm-blooded vertebrates it is the only sterol

found, but in lower animals and insects other variant forms are present. Cholesterol never occurs in plants. Schoenheimer (1929) showed that plant sterols are not the mother substances of cholesterol, and his and other work has demonstrated without question that cholesterol is synthesized (see Chapter V).

In animal tissues, cholesterol occurs in highest percentage in the white matter of the brain, where it is present to the extent of about 14 per cent of the dry weight. In gray matter, its value is 6 per cent. In other tissues, the amounts found are much smaller; for example, in kidney it is 1.6 per cent, in spleen 1.5, in skin 1.3, in liver 0.93, in the mammary glands 0.70, in whole blood 0.65, in heart muscle 0.65, in smooth muscle 0.55, in diaphragm 0.35, and in skeletal muscle 0.25 of the dry weight. In fetal muscles, it is present in larger percentages than in adult muscles. For example, in the skeletal muscle of a new-born rabbit, it was found to be 0.66 per cent of the dry weight, which is nearly three times what it would be in the same muscle of the adult.

Cholesterol is associated physiologically with the fats. Chemically it is not related to them. It is, however, related to a great variety of substances found in plants and animals; but so far as is known at present, it cannot be transformed into these substances in the living organism, nor can these substances be made into cholesterol. Thus, it is closely related chemically to the various plant sterols, and yet the plant sterols are taken into the animal body and pass out again without being changed into cholesterol to any measurable extent, even though the animal organism may find it necessary to manufacture cholesterol for its needs. It is a constituent of the bile along with the bile acids, and yet its presence or absence in the food apparently has little relation to the amount of bile acids formed.

Structure. The structure of cholesterol ($C_{27}H_{46}O$) according to Windaus (1932) is as follows:



This formula indicates the presence of only one double bond, which is in

agreement with the iodine absorption value. The nature of the nucleus is now quite well known.

Physiological properties. Cholesterol is absorbed into the animal body by way of the intestine and requires the simultaneous absorption of fat and the presence of bile salts. During the absorption, it is partly esterified with the fatty acids and appears in the lymph of the thoracic duct, partly as free cholesterol, partly as cholesterol esters. When fat alone is fed, some cholesterol always appears in the chyle, and the cholesterol for that purpose must then be supplied during the absorption of the fat, possibly from the cholesterol in the bile. It passes into the blood stream, blood plasma normally containing two-thirds of its total cholesterol in the form of cholesterol esters of the fatty acids, while the corpuscles, like other body cells, contain only free cholesterol. The fatty acids in combination with cholesterol in the blood plasma are relatively highly unsaturated, more so than are the fatty acids in combination in the fat or in the phospholipid.

When cholesterol is fed in excess, a considerable portion is excreted by way of the intestine. It can be reabsorbed after excretion into the intestine; and a mechanism for preventing reabsorption of undesired sterol appears to be its reduction to dihydrocholesterol, which is then changed over into the isomeric coprosterol, neither of which is absorbable from the intestine. Some of the excess cholesterol can be burned. There are occasional human beings in whom excretion of cholesterol is defective, and in these individuals cholesterol may collect in the blood, reaching values many times the normal level. It may be deposited in various places on the outside of the body as nodules in the skin, or in the joints. If such individuals are put on a vegetable diet, which contains no cholesterol, the high values in the blood in some cases sink to a normal value, indicating destruction of the cholesterol, probably by combustion (Schoenheimer, 1933). However, all cases do not respond to the vegetable diet (Sperry and Schick, 1936). Certain herbivorous animals, of which the best-known example is the rabbit, have little power to excrete cholesterol, and when fed in excess, it accumulates in the blood and the tissues in a way similar to that described in humans.

As cholesterol is a nonconductor of electricity, it is a good dielectric or insulator, and would theoretically be useful as the dielectric in an electric condenser. On this basis, the high content in the nerves may serve the purpose of insulating the fibers and so making stimuli specific. Its lack would result in the spread of impulses, confused thought, inaccurate movements, and so on.

Among the muscles, smooth muscle has the highest content of cholesterol, cardiac muscle next, and the skeletal muscle lowest (Bloor, 1936).

Just what this means in connection with the muscle is not known. It seems to be related to the automatic character of muscle contractions; the heart and smooth muscles, which have a much higher cholesterol content than skeletal muscle, contract automatically, whereas the skeletal muscle does not automatically contract.

Chemical and physical properties. Cholesterol is insoluble in water, sparingly soluble in cold alcohol, readily soluble in hot alcohol and in fat solvents generally. Its melting point is about 148°C. It is levorotatory, the rotation varying somewhat with the solvent. For ether it is $[\alpha]_D^{15°} = 31.1$ (Hesse, 1878). On exposure to light and air, it slowly undergoes changes; its melting point is lowered, its solubilities are changed, and its color reactions rendered indefinite.

The iodine absorption value of cholesterol, on the basis of its formula, is 65.8. With Hübls' reagent, it gives practically theoretical values (67 and 68 as compared with the theoretical, 65.8). Wijs and Hanus solutions give erratic results. The Rosenmund-Kuhnhenn pyridine sulfate-bromine reagent gives theoretical values. Ralls (1933) has contributed a study of the conditions necessary for the quantitative absorption of iodine by cholesterol, showing that the reaction is influenced by temperature and by the solvent used, and that carbon tetrachloride is a better solvent than chloroform, which is the one ordinarily used. Yasuda (1937) reported that although the Rosenmund-Kuhnhenn reagent is accurate for cholesterol, it gives too high values for ergosterol. Substituting iodine for bromine in the reagent results in good values for ergosterol, but the reagent does not act on cholesterol. Changing the medium to 3 parts methanol and 1 part acetic acid results in good values for both cholesterol and ergosterol, using the Rosenmund-Kuhnhenn reagent.

Although cholesterol is a hydrophobic colloid, it can impart to fats or oils the ability to take up considerable quantities of water, presumably because of its tendency to form water-in-oil emulsions. This peculiarity may explain the curious relation between cholesterol and water content in tissues noted by Mayer and Schaeffer (1914), as well as its protective effect against hemolysis of blood corpuscles.

Mention of other sterols is purposely omitted, since they have only a slight, if any, relation to the fatty acids and their biochemical behavior.

Bases

Several bases are found combined with the fatty acids in the various lipids. The commonest ones are *sphingosine*, a C_{18} compound having the formula, $CH_3.(CH_2)_{12}CH{=}CH.CHOH.CHOH.CH_2NH_2$; *choline*, CH_2-$OH.CH_2.N(CH_3)_3OH$; and *colamine* (aminoethyl alcohol), $NH_2.CH_2$.

CH_2OH. Sphingosine and choline are found in sphingomyelin, sphingosine in the cerebrosides, choline in lecithin and sphingomyelin, and colamine in cephalin. In addition to these, *betaine* has been found as the base in the phospholipids of *Aspergillus oryzae* (Grafe and Magistris, 1925) and *ammonia* in the phospholipid of tubercle bacilli. In calcium phosphatidate, a compound closely related to the ordinary phospholipids which has been described by Chibnall and Channon (1929), the base (choline or colamine) is replaced by *calcium* or *magnesium*.

Hydrocarbons

The unsaturated hydrocarbon, squalene ($C_{30}H_{50}$), is found in some fish liver oils (shark) and seems to replace the unsaturated acids to some extent. Other hydrocarbons of from 18 to 35 carbons have been found in plant waxes and in fish liver oils (Tsujimoto, 1916).

Split products

Incompletely hydrolyzed products of the lipids are sometimes found in tissues. Thus lignoceryl sphingosine was found by Fränkel and Bielschowsky (1932) and cerebronyl sphingosine by Klenk (1926). Partially hydrolyzed glycerides apparently do not occur.

Physico-Chemical Conceptions of the Lipids

Degkwitz (1931) analyzed the literature which has accumulated on the mode of functioning of the cell lipids, paying especial attention to the antagonism between phospholipids and cholesterol in cellular metabolism. Although much of the data is indefinite and contradictory, the results as a whole leave little doubt regarding the essential antagonism of these two substances in cellular physiology and the importance of a study of the balance of the two in physiological phenomena. For detailed information the reader is referred to the article itself.

Considerable attention is paid to the phospholipid-cholesterol balance in discussion of blood and tissues in Chaps. III and IV. Theorell (1930) discusses the general relations of phospholipid in blood and the antagonistic effect of phospholipid and cholesterol on hemolysis and sedimentation. Lewis with his co-workers (Price, 1933) deals with the surface effects of cholesterol and lecithin in relation to the isoelectric point of lecithin. Affonskii (1928) and Magistris (1932) find that cholesterol aids the diffusion of acids and alkalies into gelatin and agar jellies, and that lecithin slows it.

Cell membranes are generally believed to contain both phospholipid and cholesterol, but there is little satisfactory evidence to support the belief. Phospholipids readily become hydrated and may perhaps be able

to aid the passage of water and water-soluble substances through cell membranes. Cholesterol and cholesterol esters, although definitely hydrophobic, have marked ability to form water-in-oil emulsions and to attract and hold water in tissues, presumably because of the latter power. Cephalin also acts as a water-in-oil emulsifier, and lecithin is an oil-in-water emulsifying agent.

Kimmelstiel (1932) found that the hydrophilic lipids, lecithin and cephalin, acted differently on the isolated frog heart. Lecithin had a positively inotropic effect and in large doses increased the force of contraction. Cephalin had a chronotropic effect and might lead to diastolic standstill. The hydrophobic lipids, cerebrosides and cholesterol, had no direct influence on contraction, although they may neutralize the effects of the hydrophilic lipids and may stimulate or revive the poisoned or fatigued heart by adsorptive removal of toxins.

The Meyer-Overton theory of narcosis postulates a lipid membrane around tissue cells which has an affinity for the narcotics. The efficiency of a narcotic generally varies with its solubility in lipid and especially in oil and with its depressive effect on the surface tension of water. These two effects are generally parallel and both tend to condense the narcotic on the surface of the cell, thus modifying the properties of the surface. The permeability of the surface is decreased and so the increase of permeability that is involved in stimulation is interfered with. The Meyer-Overton law applies only to the aliphatic narcotics and morphine and other basic narcotics do not follow the law. Any theory of a lipid membrane must allow for the ready passage of water, and this involves the solubility of water in the membrane. A membrane containing a considerable proportion of phospholipid would allow the passage of water. Clowes (1916) has offered the idea that the cell membrane is made up of a mixture of oil-in-water and water-in-oil emulsions and that the permeability is dependent on the balance of these emulsion systems.

Methods of Examination of Tissue Lipids

Macromethods

Discussion

To determine the amount and nature of the lipid constituents of tissues, the efforts of the investigator are directed to the extraction of the lipids from the tissues and fluids in as nearly unchanged form as possible, the isolation of the individual lipids, and the determination of the nature and amount of the fatty acids and other substances which they contain. Regarding the fatty acids, it is desirable to know the length of chain, the degree of unsaturation, and the relative amounts of each present.

The tissue lipids which have been extensively studied are the phospholipids, lecithin and cephalin; neutral fat; and cholesterol and its esters. Sphingomyelin and the cerebrosides are normally present, except in nerve tissue, only in small amounts, and have been given relatively little attention.

Hydrolysis. Lipids in tissues are rarely free. They are confined in cells and are also combined physically or chemically with other tissue constituents. When a knowledge of only the gross lipid content is desired, processes involving hydrolytic destruction of the whole tissue may be used. Thus, the tissue may be destroyed by the use of strong alkali and the fatty material extracted from the hydrolysate. This procedure has the disadvantage of giving very little information regarding the lipids as they exist in the tissue and also may result in some alteration in the chemical nature of the product (Lemeland, 1921b, 1922, 1923). Two fractions are obtained, the fatty acids and the "unsaponifiable matter," the latter consisting of the sterols and a number of unknown substances, part of which are probably related to the sterols (Lemeland, 1921a) and part are apparently altered fatty acids. The procedure was first made use of for quantitative purposes by Liebermann, was developed in detail by Kumagawa and Suto (1908), and was later modified by Lemeland (1921b, 1922, 1923).

A method formerly in use for liberating the fats by digestion of the tissue with enzymes, such as pepsin-hydrochloric acid, is no longer much used (Rosenfeld, 1928). A recent suggestion of the use of bacteria (*B. delbruickii,* an anaerobe found in malt) for removing the protein and carbohydrate of a tissue and so freeing the fat offers an interesting alternative (Beckman, 1930).

The lipids are not equally readily hydrolyzed, and advantage is sometimes taken of their difference in this respect. For example, cholesterol esters are difficult to hydrolyze, and Fex (1920) has used this fact to separate cholesterol esters from the other esters found in the natural lipids. He found that digestion with 2 per cent alkali does not affect cholesterol esters, while it does hydrolyze the other fatty esters. Our experience with this procedure has not been satisfactory, since some of the cholesterol ester is hydrolyzed and hydrolysis of the fats is incomplete. A more promising procedure for the differential hydrolysis of these esters has been worked out by Kelsey (1939b) in this laboratory. It is based on the fact that although the castor bean lipase has no action on cholesterol esters, it hydrolyzes ordinary fat easily. Acids have also been used for differential hydrolysis, as, for example, the procedure of Zelinsky and Zinzadze (1926) and of Fischer (1926).

Dehydration and extraction. Tissue lipids are always accompanied by a great deal of water. Since they are mostly insoluble in water and

must be dissolved in solvents most of which do not mix with water, one of the first steps taken in the examination of tissues for lipid content is dehydration. Since the lipids are often chemically unstable, precautions must be taken to avoid chemical change during the dehydration and the subsequent extraction and examination. Earlier methods of dehydration consisted in drying in air at various temperatures. They were faulty because of: (1) oxidation, (2) other chemical changes due to enzymes or temperature (hydrolysis), (3) enclosure of the lipids in an impervious mass of dried protein. Drying in an atmosphere of inert gas avoided oxidation, but did not obviate (2) and (3). Freezing, pulverizing the frozen tissue, and drying in vacuum while frozen has been suggested from time to time, and theoretically is an excellent method; but because of technical difficulties it has been slow of adoption. A combination of this procedure with other methods has recently been suggested by Osato and Heki (1930).

Dehydration with cold acetone or alcohol has been employed for many years and has many advantages, but has the disadvantage that both alcohol and acetone are lipid solvents and necessitate recovery of the dissolved lipids. This difficulty is not serious and may be turned to advantage. Alcohol seems to be gaining in favor because of its more universal solvent powers. Acetone does not dissolve all the lipids, but this fact is considered by some to be an advantage because it thereby brings about a preliminary separation. The separation is, however, not complete because of the mutual-solvent action of the lipids on each other.

A procedure which combines the avoidance of chemical and enzymic changes after death, dehydrates, and at the same time extracts, is that used by Javillier and Allaire (1926). The tissue is transferred immediately after removal from the animal into ten times its weight of boiling alcohol in which it is boiled for one-half hour; then it is ground and the extraction is completed with alcohol and ether. The procedure has the disadvantage that some tissues, *e.g.*, muscle, treated in this way become so tough that grinding is difficult.

A satisfactory compromise solution of the problems involved consists in dehydrating and extracting the tissues with solvent after they have been finely divided by hashing or grinding. By this procedure, the lipids are removed from the tissues in approximately the form in which they exist there. In the experience of the writer, the most satisfactory general solvent is boiling alcohol. It has two disadvantages: it extracts other substances than the lipids, and the heat may alter some of the more labile compounds. Nevertheless, it seems superior to any other single solvent for the purpose, since it can be applied directly to the tissue without previous drying and because it penetrates the tissue readily,

breaking up whatever loose combinations there may be between lipid and other cellular constituents. .Cold alcohol, while it takes longer and gives a less complete extraction, is preferred by many because there is less danger of decomposition of the lipids.

Other fat solvents may be used for special purposes, *e.g.*, acetone to remove cholesterol and free fat, leaving most of the phospholipids. Solvents such as ether and chloroform extract relatively little from fresh tissues because they fail to penetrate, and while they do better with dried material, the extraction is generally not complete. They are useful in completing the extraction after alcohol.

Separation. Isolation of the individual lipids is often a matter of great difficulty, and procedures cannot be described in a brief review. The reader is therefore referred to special articles.[2] Separation of the members of the complex mixture of lipids is accomplished mainly by taking advantage of differences in solubility, but this, of itself, is rarely sufficient because other factors interfere. These disturbing factors are intersolubility of the compounds, oxidative and chemical changes, and intramolecular rearrangements. It takes time and various forms of treatment to overcome the intersolubility; and while this is going on, oxidation and chemical changes are difficult to avoid. In the attempt to obtain a pure compound free from other fatty substances, the result often is an impure compound due to changes during the separation. Elaborate precautions must be taken to prevent oxidative and other chemical changes brought about by the oxygen of the air and the solvents used. Operations are carried out in an atmosphere of an inert gas, such as carbon dioxide or nitrogen, and the reactions (pH) of the fluid and its temperature are closely controlled. Hence it can be readily understood that separating the lipids in pure form is a difficult procedure. It is more convenient and often none the less accurate to use indirect means of getting at the content of various compounds in the tissues. As an illustration of the factors involved in a study of the lipids of tissues, the following procedure which is in use in this laboratory is outlined:

Procedure

The tissue is obtained from the animal in as fresh a condition as possible, preferably immediately after death. (For certain purposes, it is desirable to free the tissue or organ from blood by perfusion with normal saline). It is finely divided or ground and, after weighing, is transferred

[2] For the phospholipids and galactolipids, MacLean and Smedley-MacLean (1927). and various articles by Levene and co-workers in the Journal of Biological Chemistry from 1917 to date. For cholesterol esters, Hürthle (1896), and Bloor (1924). For cerebrosides and phospholipids, Thierfelder and Klenk (1930). For general examination of tissues, Fränkel (1925).

for dehydration to a vessel containing three volumes of 95 per cent alcohol and well stirred. It is left in this liquid two or three hours with occasional stirring, after which the solid material is separated by filtration through a Büchner funnel. The liquid is saved and the dehydrated residue is completely extracted with hot alcohol. An extractor which has been found very effective is that shown in Figure 1, which is a modification (Sperry, 1926) of the Clark extractor. Its efficiency results from the fact

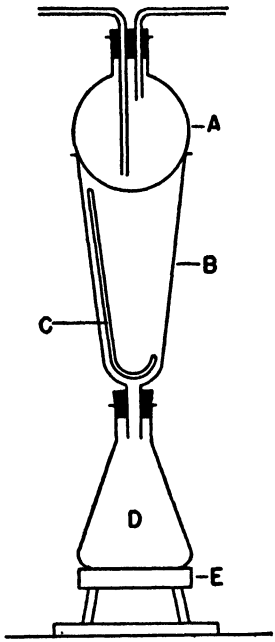


FIG. 1.

Continuous extraction apparatus. *A*, round bottom flask used as condenser; *B*, pharmacist's percolator; *C*, rod or tube to prevent too close fit of the linen bag to the wall of the percolator; *D*, flask containing the extracting fluid; *E*, electric heater. For use the finely ground material is placed in a linen bag in the percolator. As extraction proceeds, the material in the bag is stirred occasionally to break up channels.

that the tissue is (1) extracted hot, (2) subjected to continuous fresh solvent, and (3) protected from the air by being in an atmosphere of the solvent. To avoid long exposure of the extracted material to heat, the solvent containing the extracted material is removed from the flask and a fresh charge of alcohol substituted every hour of the three hours' extraction. At the same time, the material being extracted is stirred to break up channels.

After extraction is complete, the extracts are combined and the solvents removed by distillation in partial vacuum at body temperature, after which the fatty material remaining in the flask as a pasty mass is rectified by solution in ether; this leaves behind most of the water-soluble impurities. The ether solution is allowed to stand in the icebox overnight and any material which separates is removed and saved for further

examination. The ether is then evaporated to small bulk in partial vacuum at low temperature and the phospholipids separated by the use of acetone and magnesium chloride, leaving the fat and sterols in the acetone solution. After purification by reprecipitation and washing, the phospholipid is separated into lecithin and cephalin by precipitation from ether by absolute alcohol, in which the cephalin is insoluble. In the acetone solution, which contains the neutral fat and free and ester cholesterol, the free cholesterol may be determined colorimetrically or by precipitation with digitonin from the whole or a part of the solution. For the determination of neutral fat and bound cholesterol, the residue is saponified, setting free the cholesterol and fatty acids contained in the fat and cholesterol esters. The freed cholesterol may be determined as before either colorimetrically or as the digitonide. The fatty acid combined with cholesterol may be calculated, and the difference between cholesterol fatty acids and total fatty acids will be the fatty acids combined in fat. For the details of procedure and the actual determination of these different constituents, the reader is referred to the original literature (Bloor, 1924, 1926, 1927, 1928b; Sperry and Bloor, 1924; Brown, 1929b; Klenk, 1932; Gardner and Gainsborough, 1934). A general discussion of the constituent compounds is included here.

Phospholipids. Lecithin, as separated according to these directions, gives values for its fatty acid content close to the theoretical and so is in fairly pure form. Cephalin does not separate as a well-defined substance. Its analysis gives values quite far from the theoretical, especially in its content of fatty acids. Thus Levene and West (1916) gave as their best value 64 per cent of fatty acid, whereas the theoretical is about 75 per cent. After the hydrolysis, it is almost impossible to free the fatty acids from nitrogen, indicating some sort of fatty acid-nitrogen combination. Therefore, it can well be said that we do not know the true nature of the so-called "cephalin." Attempts to measure it indirectly by determinations of the α-amino nitrogen, as done by Schmitz and Koch (1930), may be of considerable assistance; but Van Slyke and associates (1935) have found the determination of cephalin by this method unsatisfactory because of the presence of other nitrogen compounds which react.

The content of sphingomyelin and cerebrosides in tissues, with the exception of the brain, is mostly unknown. In other tissues, these substances are present ordinarily in such small amounts that their separation is a matter of difficulty, the more so since they are soluble in other lipids. Values for sphingomyelin in various tissues are given by Thannhauser and associates (1939).

Cholesterol. The determination of the cholesterol content of tissues does not offer special difficulty, but there is a source of uncertainty

which is not always appreciated, that is, that the values obtained, whether determined by the digitonin precipitation or by the colorimetric method, are to an unknown extent affected by other substances which, respectively, are precipitated by digitonin, or react with the color reagents. That this is a real difficulty is well shown by the results of Anderson (1927a), who found in the unsaponifiable portion of beef plasma substances other than cholesterol, which gave either a precipitate with digitonin or a color with the Liebermann-Burchard reagent or both.

Cholesterol esters. In some tissues, mostly pathological, and also in blood plasma, cholesterol esters are present. Their determination presents some difficulty. They are not precipitated by digitonin and are difficult to saponify. The prolonged treatment with alkali necessary to insure their complete saponification may result in changes in the cholesterol which affect its determination.

Fats. In most tissues, the only fatty acid esters which are present and soluble in ether or petroleum ether are the fats, cholesterol esters, and phospholipids. In most tissues, the cholesterol esters are normally present only in traces. Hence, after the phospholipids are separated, the fatty acids present may be safely referred to as fats.

Fatty acids. In spite of a great amount of effort on the part of chemists, the separation and identification of the various fatty acids which are the essential characteristic constituents of the lipids are still far from satisfactory. The large number of different acids present, their intersolubility, the fact that they are often closely situated members of homologous series, and the fact that the highly unsaturated members often polymerize and go through isomeric changes in the process of isolation and identification all make the problem of their examination difficult. Examples of the difficulty in dealing with these substances are given by Brown (1929a, b) in his studies of arachidonic acid, and by Knauss and Smull (1927) with linolenic acid. Yet it can readily be appreciated that it is just these unsaturated acids which are of the greatest physiological importance because of their lability.

The separation of solid from liquid acids is accomplished by making use of the different solubilities of their lead salts in alcohol or ether by the Twitchell process or some modification of it. The separation is not complete, but it is good enough for many purposes. The solid acids are generally the saturated acids and the liquid acids the unsaturated, but there are some exceptions. For example, elaidic acid, an isomer of oleic acid, separates with the solid acids; advantage was taken of that fact by Sinclair (1936) to use elaidic acid in studies of fatty acid behavior. Fractional distillation in specially constructed stills has been found very

useful in separating the fatty acids of differing molecular weights (Bosworth and Brown, 1933).

Micromethods

Because of the convenience and often the necessity of working with small amounts of tissues and fluids, quantitative methods adapted for this purpose have been devised for many of the tissue lipids. Unlike the procedures for use with large amounts of material (macro or gross methods) the final determination is generally not gravimetric. Microbalances which are accurate to a hundredth and, in skilled hands, to a smaller fraction of a milligram are available, but these balances require practice in operation and take considerable time, so that most of the micromethods used in biochemical investigations have been devised to use other means of measurement. In general, it can be said that, although these micromethods are more rapid than the corresponding macromethods, much more depends on practice, skill, and what may be called the analytical instinct of the individual worker. For these reasons, it is desirable for each worker with micromethods to evolve and standardize a technic for carrying out a procedure, to which he must then rigidly adhere. In the field of the fatty substances, suitable methods for the microdetermination of the more common constituents of tissues and also of fluids are available, and sufficiently accurate determination of the fatty constituents in fractions of a gram of tissue are possible. Because of the relatively large amounts of solvent and other reagents which it is possible to use, extraction, saponification, etc., can be done more quickly and also more completely than when large amounts of tissue are used, with the result that the time is greatly shortened with little sacrifice of accuracy. Indeed, since the time and consequent exposure to oxidation is less, the microprocedure may actually be more accurate. The technic of the microdeterminations of the fatty substances in tissues can best be understood by the description of actual determinations, and for this purpose, those used in this laboratory will be described because of familiarity with them and hence the proportionately better understanding of the principles underlying them. Full details of these methods have been published and the reader is referred to the original article (Bloor, 1928a). The same substances are separated and determined as in the macroprocedures, *i.e.*, phospholipid, free and bound cholesterol, and neutral fat (by difference); also the fatty acid content of the constituents, the iodine number, and mean molecular weight (by titration) of the fatty acids. With larger amounts of the extract, the fatty acids may be separated into solid and liquid fractions, and the mean molecular weight, iodine number, and polybromide number of the liquid fatty acids can be determined. The

amount of cephalin in the lecithin-cephalin mixture may be determined with a fair degree of accuracy by a determination of the α-amino nitrogen in the hydrolysis residue. It is, however, better estimated by difference, after determining the lecithin by its choline content.

Illustrative procedure

Extraction. For blood and fluids, extraction consists in delivering the measured material slowly into an excess of a mixture of alcohol and ether so that the protein is precipitated in a finely divided form. Because of the fine state of division and the excess of solvent, extraction is rapid, and it is necessary only to bring the mixture to the boiling point in order to accomplish even distribution of the lipids through the mixture. If then the mixture is cooled and made to volume, an aliquot represents the corresponding fraction of the fatty substance contained in the material examined, and, since the weight is known, its lipid content is easily calculated. It should be emphasized that this process does not require complete extraction of the lipid from the tissue material, but only its even distribution throughout the mixture of tissue and solvent, which is easier to accomplish than complete extraction. About 25 volumes of solvent to 1 volume of fluid should be used (Boyd, 1936). A very good extraction may be made by letting the mixed material stand in the cold for about an hour, or until convenient to proceed with the next step.

For tissues, the fine state of division required for a good extraction must be obtained in other ways. Drying is undesirable because of the danger of oxidation and the formation of inextractable masses of tissue protein. Freezing and drying while frozen would undoubtedly be a good procedure, but the process is difficult even in the best equipped laboratories and is beyond the facilities of most. A satisfactory procedure for general use is to grind the finely mixed material with clean sand. By this means, the tissue can be reduced to fine form quickly and the finely ground material, together with the sand, can then be readily extracted. Since the state of division is not as fine as in fluids, the extraction must be more prolonged and energetic. However, as compared with the time taken for macromethods, the process is still relatively short. Boiling for short periods with alcohol, repeated three times, and then twice with ether,[3] gives a satisfactory extraction. The constituents of the lipid mixture dissolved in alcohol can be separated and determined in much the same way as in the macroprocess. The extraction and isolation of the separate lipids are carried out as follows:

The amount of tissue taken and the final volume of solvent used will depend on the amount of lipid present. For example, in working with

[3] Ether, wherever used, must be free of peroxides.

striated or smooth muscle which contains about 0.6 per cent of phospholipid, 8 grams of muscle in 200 cc. of extract would yield 25-cc. aliquots containing about 6 mg. of phospholipid, which is a convenient amount to use. For heart muscle, which contains twice as much phospholipid, 3 or 4 grams would be adequate; for liver with 2.5-3 per cent phospholipid, 1 or 2 grams, etc.

Isolation. The alcoholic solution contains much which is not lipid, and so must be rectified. This is best done by evaporating the alcohol with precautions to avoid oxidation[4] until only a few droplets of liquid are left, and then extracting the residue with hot petroleum ether by gently boiling with the solvent for about half a minute. The solvent is poured off into a 15-cc. centrifuge tube and the moist extraction residue reheated for a few minutes on the water bath to break up emulsions, but the heating should not be carried to dryness. The residue is then extracted twice more with boiling petroleum ether, the whole volume of extract being adjusted to the volume of the centrifuge tube. This treatment gives a good extraction of the lipids, and it leaves behind most of the non-lipid substances, salts, sugar, etc., which had been in solution in the alcohol.

Phospholipids. For the determination of the phospholipids, the petroleum ether is first centrifuged to clear it and after transfer to a clean 15-cc. centrifuge tube is evaporated to a conveniently small bulk (2 cc.), making use of a boiling rod to promote even boiling. Acetone to nearly fill the tube and 2 to 4 drops (about 1 drop per mg. of phospholipid) of a 4.5 per cent alcoholic solution of magnesium chloride are added, and the material is stirred. This treatment is sufficient to produce a practically complete precipitation of the phospholipid if the amount is above 2 mg. Ordinarily, an aliquot of the extract containing 4-6 mg. of phospholipid is used. The precipitated phospholipids are collected by gentle centrifugation and may then be dissolved in moist ether for analysis. With moist ether, much of the sphingomyelin remains undissolved and may later be dissolved in chloroform for measurement. Three ways are available for the measurement of the amount of total phospholipid: (1) oxidation using standard potassium dichromate with sulfuric acid and a catalyst (Bloor, 1929); (2) indirectly by measurement of the phos-

[4] Various devices have been suggested to avoid loss during evaporation, thus: evaporation in vacuum (Ellis and Maynard, 1937), evaporation below 60°C. (Kirk, Page and Van Slyke, 1934), and in inert gas (Man and Gildea, 1932). In our experience, it is sufficient to evaporate in a small Erlenmeyer flask fitted with a small close-fitting watch glass. As the material is heated, the alcohol evaporates, driving out the air which is prevented from reentering by the watch glass, which acts as a flutter valve, loosely sealed by the condensed alcohol. Heating should be carried only to absence of alcohol and not to dryness, which renders the lipids difficult to extract.

phoric acid which it contains, which may be done by several quite satisfactory methods: colorimetric (Fiske and Subbarow, 1925), nephelometric (Bloor, 1918), and microgravimetric (Pregl, 1924); or (3) determination of the fatty acids contained in the phospholipid, either by the oxidative procedure as for phospholipid, or by titration, which gives good results when properly performed. Artom (1932) found that this titration procedure gives more accurate values than the determination by lipid phosphorus which in his hands gave values averaging 18 per cent too high. As has been noted (Le Breton, 1921), the determination of phospholipid by calculation from its phosphorus content (lipid phosphorus) is liable to considerable error.

After the precipitation of the phospholipid, there are left in the acetone the other fatty substances found in the tissue or fluid. These consist ordinarily of fat and sterols, mainly cholesterol, and the solution may be used for the determination of these substances.

Total fatty acid. Sufficient extract for a determination (enough to yield about 0.5 mg. of cholesterol and 4 mg. or more of fatty acid) is measured into a small flask, 2 drops of 40 per cent sodium hydroxide are added, and the whole evaporated almost to absence of alcohol, which is shown by the appearance of foaming. The last traces of alcohol are blown out by a gentle current of air, after which the mixture is acidified with dilute sulfuric acid (1 part concentrated sulfuric acid and 3 parts of water), warmed, and gently shaken to set free the fatty acids. Petroleum ether is added to the solution at once and the material mixed by gentle rotation. The fatty acids and cholesterol dissolve quickly, and the petroleum ether is decanted into a small graduated flask (ordinarily 25-cc.). Because of the difference in the surface properties of the two liquids, the petroleum ether may be decanted almost quantitatively from the watery residue in the flask. After removal of the petroleum ether, the water in the flask is distributed over the inside surface of the flask by strong shaking so as to reach any material which has splashed up on the sides. It is then heated for a few minutes and the extraction with petroleum ether repeated twice more, the last time with strong shaking using enough solvent to complete the filling of the graduated flask. The flask is then cooled and the contents made to volume. The extract contains all the fatty acid and cholesterol.

The total fatty acid can be determined in the petroleum ether solution either by titration with alkali after washing free from water-soluble acid, or by oxidation, as is done in this laboratory (Bloor, 1928a). The oxidative method requires less material. Cholesterol can be determined by methods given below under "Free and ester cholesterol." In this laboratory, it is found convenient to determine oxidatively the total material

(fatty acid plus cholesterol) in one aliquot of the petroleum ether extract, then cholesterol colorimetrically in another. Since we know the oxidation factor of the cholesterol, its dichromate value can be subtracted from the total titration value and the difference will give the value for the fatty acid. Strictly speaking, this is not quite correct because of the presence in the extract of unsaponifiable substance other than cholesterol. Since this other unsaponifiable substance is derived in part from the fatty acids, the error is not great; but, if desired, this material may be determined by the method described by Yasuda (1931a) and the value subtracted from the fatty acid value, the difference giving the true fatty acid value.

Free and ester cholesterol. A variety of methods for the determination of free and ester cholesterol is available, of which the most recent one from this laboratory is that of Kelsey (1939a). In a procedure proposed by the writer and Knudson (1916) many years ago, saponification is avoided. There is always a hazard in making a saponification of the cholesterol esters because of changes produced in the cholesterol by the alkali used in the saponification procedure, which is necessarily drastic, since the esters are much more difficult to saponify than either fat or cholesterol. The alkalinity appears to have much less effect on the color-producing properties of the cholesterol than on its ability to precipitate digitonin.

For the determination of free and ester cholesterol, an aliquot of the original alcoholic extract is taken and the free cholesterol precipitated from it by digitonin. After evaporation to dryness, the cholesterol ester which remains is extracted by petroleum ether from the dried mass. The petroleum ether extracts are evaporated and the residue is taken up in chloroform, after which colorimetric measurement is made as usual. The value obtained is that for the cholesterol combined as ester. The total cholesterol may be determined in another aliquot of the alcoholic extract by evaporation to dryness and taking up in chloroform. When the value for ester cholesterol is subtracted from this, the value for free cholesterol is obtained. Free cholesterol may also be determined directly from the digitonin precipitation mentioned previously. The digitonide may be measured gravimetrically with the microbalance, oxidatively by the dichromate oxidation mixture, or colorimetrically by the procedure devised by Schoenheimer and Sperry (1934). The colorimetric method applied directly to the extraction residues generally gives results 10-20 per cent higher for total cholesterol than the digitonin precipitation method. One reason which has been given is that of Reinhold (1935) that the color from the ester develops faster and more completely than that from the free cholesterol. If this were the case, it would be expected that the cholesterol as ester percentage would be especially high by the

Bloor-Knudson method. Actually, it is lower than those obtained by Sperry using the digitonin-colorimetric method. The complete story of cholesterol determinations in blood is probably not yet written. Neither the colorimetric nor the digitonin precipitation method is specific, and it is likely that neither shows cholesterol alone. Since the digitonin method gives lower values, it is often assumed to be more nearly correct. The technic for a successful determination by digitonin is difficult, with considerable chance of loss by decomposition of the digitonin-cholesterol complex or of loss in the wash liquids. Onizawa (1928), in a comparison of the two procedures, found that the colorimetric method compared favorably with the digitonin precipitation method in whole blood plasma, liver, and spleen, but not in other tissues.

Neutral fat. Knowing the amount of fatty acid combined with cholesterol and with phospholipid, it is now possible to arrive at a value for the neutral fat as follows. From the total fatty acid, as determined, subtract the fatty acids in combination in the phospholipids (two-thirds of the weight of the phospholipids) and the amount of fatty acid in combination with cholesterol as ester (approximately two-thirds of the weight of the combined cholesterol). This gives a value which represents residual fatty acids, but since it comes largely from neutral fat, it is so called.

By this system of analysis, there are obtained from one sample of blood or tissue, values for the following substances: phospholipid, free cholesterol, ester cholesterol, unsaponifiable substances other than cholesterol, and fat, which together give a good idea of the lipid composition.

Iodine number. The micromethods for iodine number determination of lipids are based on the macromethods and have their faults. A micro-iodine number based on the Hanus macromethod has been worked out by Gibson and Howard (1923) who use it on the total lipid of blood including cholesterol. Yasuda (1931b) found that the Hanus method was inaccurate for cholesterol and certain of the fatty acids. He adapted the Rosenmund-Kuhnhenn pyridine-sulfate-bromine solution for use as a micromethod with the lipids of blood and tissues, and his procedure has been found satisfactory.

Discussion

Additional determinations. Some constituents not mentioned in the procedure just outlined are, for example, sphingomyelin and cerebrosides. However, except in the brain and nerves, some samples of blood plasma, and certain pathological tissues, these substances are present in such small amounts that they may be neglected. A method for measurement of sphingomyelin has been offered: that of Thannhauser and Setz (1936) by precipitation as the reineckate. A method for the cerebrosides de-

pending on the measurement of the galactose content (Kirk, 1938) has been devised.

A complete system for the microdetermination of the lipids in blood, making use of the Van Slyke manometric apparatus, has been offered by Kirk, Page and Van Slyke (1934). Sources of error in these determinations have been pointed out by Christensen (1939) and by Folch and Van Slyke (1939).

Digitonin precipitation for cholesterol. Windaus (1910) devised a method for cholesterol depending on its precipitation as a compound of digitonin, which is dried and weighed. The weight of the digitonide divided by four gives the weight of cholesterol (cholesterol digitonide $\times$ 0.25 = cholesterol). The digitonide decomposed on boiling with xylene so that the digitonin can be recovered, a fact of importance because of its high cost. Windaus did not claim that the reaction was specific for cholesterol but named several other substances which would also give precipitates with digitonin.

This fact is lost sight of by many of those using the method, but attention has recently been called to it by the work of Anderson (1927a), who found in the "unsaponifiable substance" from beef plasma a considerable amount of material not cholesterol which precipitated with digitonin. Various details of the original Windaus method have been intensively studied (Thaysen, 1914b; Dam, 1928), the result being an increased precision.

Von Szent-Györgyi (1923) devised a micromethod for measuring the digitonide by oxidation with chromic acid, which was later modified by Tominaga (1925). Their procedures do not result in complete oxidation of the compound, a fact which introduces an element of uncertainty. A method making use of complete oxidation suitable for use with 1 mg. of cholesterol was devised by Okey (1930) and later modified by Yasuda (1931a). Digitonin microprecipitation methods making use of the microbalance have been devised by von Szent-Györgyi (1923), by Mancke (1931), and by Mühlbock and Kaufmann (1931). The digitonin precipitation method in its various forms is regarded by most workers as the most accurate of all the methods for cholesterol, which may be the case; but the effect of alkali in saponification and the possibility of other compounds than cholesterol being precipitated are objections to complete faith in it. Another note of warning is sounded by Schoenheimer and Dam (1933) who find the digitonide much less stable and insoluble than is ordinarily supposed, and low values for cholesterol may be obtained as the result.

Colorimetric methods for cholesterol. Because of its convenience, the determination of cholesterol colorimetrically is the method most com-

monly used. The color reaction mainly made use of is that of Liebermann, modified by Burchard, and consists of treating the cholesterol in chloroform solution with acetic anhydride and a small amount of sulfuric acid. The color produced is a green, and is measured by means of a standard color produced under the same conditions from pure cholesterol. Many methods making use of this reaction are available, the earliest being that of Autenrieth and Funk (1913). Bloor (1916), Myers and Wardell (1918), Leiboff (1924), Ling (1928), Grigaut (1933), and others have used modifications of this technic. Aside from modifications intended to bring about easier extraction from biological material and to give greater precision in use, the recent procedures are much the same as the original. Their great number indicates that the colorimetric procedure is still not satisfactory. When used with pure cholesterol, the results are good by either the digitonin or the colorimetric methods (Mueller, 1916; Gardner and Williams, 1921). When used with blood or other biological material foreign substances which react, incomplete extraction, etc., affect the results by both methods. It is the current belief that the colorimetric method is affected by foreign substances to a greater extent than the precipitation method (Anderson, 1924).

Anderson (1924) has given considerable attention to the sterols in plants and animals, attempting to distinguish the various substances from one another. He found that the Liebermann-Burchard reaction is not given by the saturated sterols. The color is formed by combination with sulfuric acid at the double bond, and this compound with the acetic anhydride gives the color. A few drops of water removes all colored substances leaving the saturated sterol in the solvent partly as the acetyl derivative and partly as the ethereal sulfate. Saponification with potassium hydroxide yields the free sterols.

Theorell and Widström (1931) found that the use of the Zeiss-Pulfrich photometer permits a correction for the variable brownish color often encountered in the colorimetric method, and the corrected values agreed well with the digitonin method. Good discussions of the relative merits of the digitonin and colorimetric methods for cholesterol are given by Gardner and Williams (1921) and by Onizawa (1928). A combination of the digitonin precipitation with the colorimetric determination on the digitonin precipitate by Schoenheimer and Sperry (1934) promises to be useful.

Titration methods for fatty acids. That fatty acids could be accurately titrated with standard alkali, providing suitable solvents were used, has been known for a long time (Folin and Wentworth, 1910; Gephart and Csonka, 1914), and recently there has been a revival of their use on a microscale. Stewart and White (1925) offered a method which

was later shown to be faulty (Long and Venning, 1932) because of the inclusion of other organic acids. Stoddard and Drury's method (1929) avoided this difficulty, and their procedure has been further modified by Man and Gildea (1932).

Artom (1932) called attention to the fact that the acidometric titration of the fatty acids had been used in Italy for a long time, discussed the sources of error in this procedure, and offered a scheme for the avoidance of these difficulties. Lemeland (1921b, 1922, 1923) had shown that during saponification by the Kumagawa-Suto procedure some of the fatty acids are oxidized to hydroxy acids which are left behind during the treatment with petroleum ether, thus lowering the fatty acid value and increasing the "unsaponifiable"; also that during the acidification for the separation of the fatty acids some condensation takes place resulting in high molecular weights. The latter difficulty is overcome by Artom by warming with excess of alkali which breaks up the condensed products, followed by back titration. Artom finds that for the determination of phospholipids (separated by acetone-magnesium chloride precipitation) saponification followed by titrimetric measurement of the fatty acids gives much better results than determination of the phosphorus, which may result in values 50 per cent too high and averages 18 per cent high.

Bibliography

Abderhalden, E., "Biochemisches Handlexikon," Vol. 3, p. 178, Berlin, J. Springer, 1911. p. 5
Abderhalden, E and Eichwald, E., *Ber. deut chem. Ges.*, **48**. 1847 (1915). p 8
Adam, N. K. and associates, a series of articles from 1921 to 1928, including: Adam, N K. *Proc Roy Soc. London*, **A99**: 336 (1921); Adam, N. K., Berry, W. A. and Turner, H. A, *ibid.*, **A117**: 532 (1927–28). p. 28
Adam, N. K., Danielli, J. F., Haslewood, G. A. D. and Marrian, G. F., *Biochem. J.*, **26**: 1233 (1932). p. 32
Affonskii, S I., *Biochem Z.*, **195**: 387 (1928). p. 36
Allaire, H., *see* Javillier, M.
Amberger, C. and Bromig, K., *Biochem. Z.*, **130**: 252 (1922). p. 10
Anderson, R. J., *J. Am. Chem. Soc*, **46**: 1450 (1924). p. 51
J. Biol. Chem., **71**: 407 (1927a). p. 43, 50
ibid., **74**: 537 (1927b). p., 14, 15
ibid., **83**: 169 (1929). p. 21
J. Am. Chem. Soc., **52**: 1607 (1930). p. 14
Anderson, R. J. and Moore, M. G, *J. Am. Chem. Soc.*, **45**: 1944 (1923). p. 31
Anderson, R. J. and Renfrew, A. G, *J. Am. Chem. Soc.*, **52**: 1252 (1930). p. 14
André, E., *Bull. soc. chim. biol.*, **7**: 974 (1925). p. 22, 23
Armstrong, E. F. and Hilditch, T. P., *J. Soc. Chem. Ind.*, **44**: 180T (1925). p. 27
Armstrong, H. E. and Gosney, H. W., *Proc. Roy. Soc. London*, **B88**: 176 (1914). p. 11
Artom, C., *Bull. soc. chim. biol.*, **14**: 1386 (1932). p. 47, 52
Artom, C. and Réale, L., *Bull. soc. chim. biol.*, **18**: 959 (1936). p. 11
Ault, W. C., *see* Brown, J. B
Autenrieth, W. and Funk, A., *Münch med. Wochschr.*, **60**: 1243 (1913). p. 51
Baer, E. and Fischer, H. O. L., *J. Biol. Chem*, **128**: 475 (1939). p 8, 11
Banks, A. and Hilditch, T. P., *Biochem. J.*, **25**: 1168 (1931). p. 3
Bauer, K. H. and Ermann, F., *Chem. Umschau. Fette, Öle, Wachse Harze*, **37**: 241 (1930); *C. A.*, **24**: 5721 (1930). p. 28
Beckman, J. W., *Ind. Eng Chem.*, **22**: 117 (1930). p. 38
Bedford, F., *see* Erdmann, E.
Belfanti, S., *Z. Immunitäts*, **44**: 347 (1925). p. 17
Bergmann, M. and Sabetay, S., *Z. physiol. Chem.*, **137**: 47 (1924) p. 8
Berry, W. A, *see* Adam, N. K.
Bertram, S. H., *Biochem. Z.*, **197**: 433 (1928). p. 23
Best, C. H. and Ridout, J. H., *J. Physiol.*, **84**: 7P (1935). p. 11
Best, C. H., Channon, H. J. and Ridout, J. H., *J. Physiol.*, **81**: 409 (1934). p. 11
Bielschowsky, F., *see* Fränkel, E.

Blix, G., *Z. physiol Chem*, 219: 82 (1933) p 21
Bloor, W. R., *J. Biol. Chem.*, 24: 227 (1916). p 51
ibid., 36: 33 (1918). p 47
ibid., 59: 543 (1924). p 40, 42
ibid., 68: 33 (1926); 72: 327 (1927). p. 42
ibid., 77: 53 (1928a). p. 44, 47
ibid., 80: 443 (1928b). p. 14, 42
ibid., 82: 273 (1929). p. 46
ibid, 114: 639 (1936). p. 34
See also Sperry, W. M.
Bloor, W. R. and Knudson, A., *J. Biol. Chem*, 27: 107 (1916) p 48
Bomer, A., *Z. Untersuch. Nahr. u Genussm.*, 14: 90 (1907) p. 6
Bolaffi, A., *Biochim. terap. sper.*, 18: 372 (1931); *C. A.*, 25. 4314 (1931). p 21
Bosworth, A. W. and Brown, J. B., *J. Biol. Chem.*, 103: 115 (1933). p. 44
Boutwell, P. W., *see* Steenbock, H.
Boyd, E. M., *J. Biol. Chem.*, 114: 223; 115: 37 (1936). p. 45
Brigl, P. and Fuchs, E., *Z. physiol. Chem*, 119: 280 (1922). p. 6
Bromig, K, *see* Amberger, C.
Brown, E. W., *Am. J. Physiol.*, 2: 306 (1899). p. 11
Brown, J. B, *J. Biol. Chem.*, 80: 455 (1928). p. 24
ibid., 83: 777 (1929a). p. 21, 22, 43
ibid., 83: 783 (1929b). p. 42, 43
See also Bosworth, A. W.
Brown, J. B. and Ault, W. C., *J. Biol. Chem.*, 89: 167 (1930) p 24
Brown, J. B and Deck, E M, *J. Am. Chem. Soc*, 52: 1135 (1930) p 24
Brydówna, W., *see* Kuhn, R.
Bull, H. B., "The Biochemistry of the Lipids," New York, John Wiley and Sons, 1937. p. 3, 31
Burr, G. O., *see* Miller, E S.
Burton, L. V., *see* MacArthur, C. G.
Butenandt, A., *J. Soc. Chem. Ind.*, 55: 753, 891, 990 (1936). p 32
Caldwell, B. P. and Piontkowski, F. A., *J. Am. Chem Soc*, 56. 2086 (1934) p. 28
Cavaignac, —, *see* Le Chatelier, H.
Chain, E., *see* Fishgold, H.
Channon, H. J., *see* Best, C. H., Chibnall, A. C.
Channon, H. J., Irving, E., and Smith, J. A. B., *Biochem. J.*, 28 1807 (1934). p 23
Chargaff, E., *J. Biol. Chem*, 125: 661 (1938). p 16
Chargaff, E. and Cohen, S S., *J. Biol. Chem*, 129: 619 (1939). p 18
Chen, K K. and Chen, A. L., *J. Pharmacol*, 49: 561 (1933). p. 32
Chibnall, A. C, *see* Jordan, R C.
Chibnall, A. C. and Channon, H. J., *Biochem. J.*, 23: 176 (1929). p. 19, 36
Christensen, H. N, *J Biol. Chem.*, 129: 531 (1939). p. 50
Clarkson, C. E. and Malkin, T., *J. Chem. Soc*, 1934: 666. p. 6
Clowes, G H. A., *J. Phys. Chem.*, 20: 407 (1916). p. 37
Cohen, S. S, *see* Chargaff, E
Collin, G and Hilditch, T P, *Biochem. J.*, 23: 1272 (1929). p. 3
Csonka, F. A., *see* Gephart, F. C.
Cummings, M J. and Mattill, H. A., *J. Nutrition*, 3: 421 (1930–31). p. 10
Dam, H, *Biochem. Z.*, 194: 177 (1928). p. 12, 50
See also Schoenheimer, R.
Danielli, J. F., *see* Adam, N. K.
Daubert, B F. and King, C. G., *J. Am. Chem. Soc.*, 61: 3328 (1939) p. 10
Deck, E M, *see* Brown, J. B.
Degkwitz, R., *Ergeb. Physiol*, 32: 821 (1931). p. 36
de Jong, H. G. B. and Westerkamp, R. F., *Biochem. Z.*, 234: 367 (1931). p 16
Deysher, E. F., *see* Holm, G. E.
Dolan, M., *see* King, E. J.
Dowell, S. T., *Okla. Expt. Sta Science*, 53: 487 (1921); *C. A.*, 15: 2300 (1921). p 28
Drury, P E, *see* Stoddard, J. L.
Eckles, C H., *see* Palmer, L. S.
Eggerth, A. H., *J. Exptl Med.*, 53: 27 (1931). p. 29
Eichwald, E., *see* Abderhalden, E.
Elderfield, R. C. and Jacobs, W. A., *Science*, 79: 279 (1934). p. 32
Ellis, G. W., *Biochem. J.*, 26: 791 (1932). p. 27
Ellis, G and Maynard, L. A., *J. Biol. Chem.*, 118: 701 (1937). p. 46
Emery, J A. and Henley, R. R., *J. Ind. Eng. Chem.*, 14: 937 (1922). p. 9
Erdmann, E. and Bedford, F., *Ber. deut. chem. Ges.*, 42: 1324 (1909). p. 24
Erdmann, E., Bedford, F. and Raspe, F., *Ber. deut. chem. Ges*, 42: 1334 (1909). p 24
Ermann, F., *see* Bauer, K. H.
Evans, R D., *see* Larson, W. P.
Färber, E., *see* Neuberg, C.
Fex, J., *Biochem. Z.*, 104: 82 (1920). p 38
Fierz-David, H. E., *Z. angew Chem.*, 38: 6 (1925). p. 10
Fischer, A, *Biochem. Z.*, 175: 449 (1926). p. 38
Fischer, H. O. L., *see* Baer, E.
Fishgold, H. and Chain, E., *Proc. Roy. Soc. London*, B117: 239 (1935). p. 16
Fiske, C. H. and Subbarow, Y., *J. Biol. Chem.*, 66: 375 (1925). p. 47
Foà, C. *Arch. ital. biol.*, 63: 229 (1915). p. 11
Folch, J, *J. Biol. Chem.*, 139: 973 (1941). p. 17
Folch, J. and Van Slyke, D. D., *J. Biol. Chem.*, 129: 539 (1939). p 50
Folin, O. and Wentworth, A. H., *J. Biol. Chem.*, 7: 421 (1910). p. 51
Fox, F. W., *see* Gardner, J. A.
Fränkel, E. and Bielschowsky, F., *Z. physiol. Chem.*, 213: 58 (1932). p. 36
Frankel, S., from Abderhalden, E., "Handbuch der Biologischen Arbeitsmethoden," 1. Abt., Teil 6, pp 1–128 Berlin. Urban and Schwarzenberg, 1925 p. 40
Fuchs, E., *see* Brigl, P.

Funk, A., *see* Autenrieth, W.
Furukubo, T., *see* Sueyoshi, Y.
Gainsborough, H., *see* Gardner, J. A.
Gardner, J. A. and Fox, F. W., *Biochem. J.*, **18**: 1058 (1924). p. 12
Gardner, J. A. and Gainsborough, H., *Biochem J.*, **28**: 1631 (1934). p. 42
Gardner, J. A. and Williams, M., *Biochem. J.*, **15**: 363 (1921). p. 51
Garner, W. E and King, A. M., *J. Chem. Soc.*, **1929**: 1849. p. 6
Gephart, F. C. and Csonka, F. A., *J. Biol. Chem.*, **19**: 521 (1914). p. 51
Gibson, R. B. and Howard, C P, *Arch Internal Med.*, **32**: 1 (1923). p 49
Gies, W. J, *see* Rosenbloom, J.
Gildea, E F, *see* Man, E B
Gosney, H. W., *see* Armstrong, H. E
Grafe, V. and Magistris, H., *Biochem. Z*, **162**: 366 (1925). p 36
Green, R. G., *see* Larson, W. P.
Greenbank, G. R., *see* Holm, G. E.
Greenbank, G. R. and Holm, G. E., *Ind. Eng. Chem.*, **16**. 598 (1924); **17**: 625 (1925). p. 9
Grigaut, A., *Compt. rend. soc. biol.*, **112**: 34 (1933). p. 51
Grün, A, *see* Halden, W.
Grün, A. and Kade, F., *Ber deut. chem. Ges.*, **45**· 3367 (1912). p 15
Grün, A. and Limpacher, R., *Chem. Umschau. Fette, Öle, Wachse Harze*, **30**: 246 (1923); *C. A.*, **18**: **536** (1924). p. 13, 15
Grün, A. and Schacht, P., *Ber. deut chem. Ges*, **40**: 1778 (1907). p 6
Grün, A. and Theimer, E, *Ber. deut. chem Ges.*, **40**· 1792 (1907). p 10
Grüntzig, W., *Z. anorg. Chem.*, **240**: 313 (1939). p. 6
Guth, F., *Z. Biol*, **44**: 78 (1903) p 5
Hafner, A., *see* Kreis, H
Halden, W. and Grün, A, "Analyse der Fette und Wachse. II Bd., Systematik Analysenergebnisse Bibliographie der naturlichen Fette und Wachse," Berlin, Julius Springer, 1929. p. 2
Halvorson, H. O., *see* Larson, W. P.
Hamsik, A., *Z. physiol. Chem.*, **59**: 1 (1909). p. 11
Hartley, P., *J. Physiol*, **36**: 17 (1907); **38**: 353 (1909) p. 23
Haslewood, G. A. D, *see* Adam, N. K.
Hausser, I., *see* Kuhn, R
Hauth, A., *see* Windaus, A.
Heki, M., *see* Osato, S.
Henley, R. R., *see* Emery, J. A.
Hepner, E., *Arch. ges. Physiol. (Pflügers)*, **73**: 595 (1898) p 11
Hesse, O., *Ann. Chem*, **192**: 175 (1878). p. 35
Hilditch, T P., *Allgem. Oel- u. Fett-Ztg*, **27**· 93, 111 (1930); *C A.*, **24**. 4176 (1930). p. 3
See also Armstrong, E. F, Banks, A., Collin, G.
Hilditch, T. P and Paul, S, *Biochem. J.*, **32**: 1775 (1938). p 4
Holm, G. E., *see* Greenbank, G. R.
Holm, G. E. and Greenbank, G. R, *Ind. Eng Chem.*, **15**: 1051 (1923). p. 9, 10
ibid., **16**: 518 (1924). p. 9
Holm, G. E., Greenbank, G R. and Deysher, E. F., *Ind. Eng. Chem*, **19**: 156 (1927). p 9
Holmes, A. D., *see* Langworthy, C. F.
Hopkins, F. G., *Biochem. J.*, **19**: 787 (1925). p. 27
Howard, C. P, *see* Gibson, R B.
Hürthle, K., *Z. physiol. Chem.*, **21**: 331 (1896). p. 11, 40
Inoue, Y., *see* Suzuki, B.
Irving, E., *see* Channon, H. J.
Jacobs, W. A, *see* Elderfield, R. C
Jamieson, G. S, "Vegetable Fats and Oils," New York, Chemical Catalog Co, (Reinhold Publishing Corp) 1932. p. 2
Javillier, M and Allaire, H., *Bull. soc. chim. biol.*, **8**: 924](1926). p. 39
Jordan, R C and Chibnall, A. C, *Ann Botany*, **47**: 163 (1933). p 19
Jukes, T. H., *J. Biol. Chem*, **107**: 783 (1934). p. 14, 16
Kade, F, *see* Grün, A.
Karrer, P. and Salomon, H., *Helv. Chim. Acta*, **9**: 3 (1926). p. 31
Kaufmann, C., *see* Mühlbock, O.
Kelsey, F. E., *J. Biol. Chem.*, **127**: 15 (1939a). p. 48
ibid., **130**: 187 (1939b). p. 12, 38
Kent, H. E., *see* Steenbock, H.
Kerr, R H. and Sorber, D. G, *Ind Eng Chem.*, **15**: 383 (1923). p. 9, 10
Kimmelstiel, P., *Arch. path. Anat. (Virchow's)*, **284**: 835 (1932). p. 37
King, A. M., *see* Garner, W. E.
King, C. G., *see* Daubert, B. F.
King, E. J., *Biochem. J.*, **28**: 476 (1934). p. 15
King, E. J. and Dolan, M., *Biochem. J.*, **27**: 403 (1933) p. 18
Kirk, E., *J. Biol. Chem.*, **123**: 613 (1938). p. 50
See also Van Slyke, D. D.
Kirk, E., Page, I. H. and Van Slyke, D. D, *J Biol. Chem.*, **106**. 203 (1934). p. 46.50
Klenk, E, *Z. physiol. Chem.*, **153**: 74 (1926). p. 36
ibid., **166**: 287 (1927). p. 23
ibid., **179**: 312 (1928). p. 20
ibid., **185**: 169 (1929). p. 24
ibid., **200**: 51 (1931). p. 24
ibid., **206**: 25 (1932). p. 42
See also Thierfelder, H.
Knauss, C. A. and Smull, J. G., *J. Am. Chem. Soc.*, **49**: 2808 (1927). p. 24, 43
Knudson, A., *see* Bloor, W. R.
Koch, F., *see* Schmitz, E.
Koch, W., *Z. physiol. Chem.*, **36**: 134 (1902). p. 20
Koch, W. and Koch, M. L., *J. Biol. Chem.*, **31**: 395 (1917). p. 20
Komatsu, S., *see* Levene, P. A.

Kraybill, H. R., *see* Shrewsbury, C. L.
Kreis, H. and Hafner, A., *Ber. deut. chem. Ges.*, **36**: 2766 (1903) p. 10
Kuhn, R., Hausser, I. and Brydówna, W., *Ber. deut. chem Ges.*, **68**: 2386 (1935). p. 14
Kumagawa, M. and Suto, K., *Biochem. Z.*, **8**: 212 (1908). p. 38
Kúthy, A, *see* Verzár, F.
Lamar, R. V., *J Exptl Med*, **13**: 1 (1911). p. 29
Landsteiner, K., *see* Levene, P. A.
Langmuir, I., *J. Am. Chem Soc.*, **39**: 1848 (1917). p. 28
Langworthy, C. F and Holmes, A. D., *U. S. Dept Agr*, *Bull*, **310**: 181, 188 (1915), *C A.*, **10**. 625 (1916). p 27
Larson, W. P. and associates (Halvorson, H. O., Evans, R D. and Green, R G.), "Colloid Symposium Monographs," Vol 3, p 152, New York, Chemical Catalog Co (Reinhold Publishing Corp) 1925. p. 29
Lawrie, J. W., "Glycerol and the Glycols," New York, Chemical Catalog Co., (Reinhold Publishing Corp.) 1928. p. 31
Lease, E. J. and associates (Lease, J. G., Weber, J. and Steenbock, H.), *J. Nutrition*, **16**. 571 (1938). p 9
Leathes, J B., *Lancet*, **208** 603, 853, 957, 1019 (1925). p 15
Leathes, J. B and Raper, H. S., "The Fats," 2d ed., New York, Longmans, Green and Co, 1925 p 1, 2, 26
Le Breton, E, *Bull soc chim biol.*, **3**: 539 (1921). p 47
Le Chatelier, H and Cavaignac, —, *Compt. rend acad sci.*, **156**: 589 (1913). p. 5
Lee, J. van der, *see* Verkade, P. E
Leiboff, S L., *J. Biol Chem.*, **61**: 177 (1924). p 51
Lemeland, P, *Compt rend. soc. biol*, **85**. 839 (1921a). p 38
Bull. soc chim. biol, **3** 134 (1921b), **4**· 300 (1922); **5**: 110 (1923). p 38, 52
Levene, P. A, *J Biol. Chem*, **18** 453 (1914) p 19
ibid., **24**: 69 (1916). p. 18, 19
Levene, P A. and Komatsu, S, *J. Biol. Chem.*, **39**: 91 (1919) p 19
Levene, P. A. and Landsteiner, K., *J Biol Chem.*, **75**: 607 (1927) p. 20
Levene, P. A. and Rolf, I P, *J. Biol Chem.*, **51**: 507 (1922a). p 14
ibid., **54**· 91 (1922b). p 17
ibid., **60**: 677 (1924a). p. 15, 17
ibid., **62**: 759 (1924b) p 14
ibid, **72**: 587 (1927a). p 15
ibid, **74**: 713 (1927b). p. 17
Levene, P. A and Simms, H. S, *J. Biol. Chem.*, **51**· 285 (1922) p. 14
Levene, P. A. and West, C J., *J Biol. Chem*, **24**. 41 (1916) p 42
Levene, P. A., Rolf, I P. and Simms, H S, *J. Biol Chem*, **58**: 859 (1924). p. 15
Lewkowitsch, J, "Chemical Technology and Analysis of Oils, Fats, and Waxes," 6th ed, Vol. 1, p. 28, 237, 239, London, Macmillan, 1921. p 5, 25
Limpacher, R, *see* Grün, A.
Ling, S M., *J. Biol Chem.*, **76**: 361 (1928). p 51
Long, C. N H and Venning, E M, *J Biol Chem*, **96**: 397 (1932) p 52
Longenecker, H. E, *J. Biol. Chem*, **129**: 13 (1939). p. 28
Lovern, J A, *Biochem J*, **30**. 2023 (1936) p 3
MacArthur, C. G and Burton, L. V., *J. Am Chem Soc*, **38**: 1375 (1916). p. 17
McBain, J. W, *see* Norris, M. H.
McBain, J W. and associates, *J Chem Soc*, **121**: 621, 1320, 1362, 2325 (1922). p 29
MacLean, H. and Smedley-MacLean, I, "Lecithin and Allied Substances, the Lipins," 2d ed, London, Longmans, Green and Co, 1927 p 1, 2, 40
Magistris, H, *Biochem Z.*, **253** 81 (1932). p 36
See also Grafe, V
Magistris, H and Schäfer, P, *Biochem Z*, **214**. 401, 440 (1929). p. 15
Malkin, T, *see* Clarkson, C E
Man, E B. and Gildea, E F, *J Biol Chem*, **99**· 43 (1932). p 46, 52
Mancke, R, *Biochem Z*, **231** 103 (1931). p 50
Marrian, G F, *see* Adam, N K
Mattill, H A, *J. Biol Chem*, **90**: 141 (1931) p. 10
See also Cummings, M J.
Mayer, A. and Schaeffer, G, *Compt. rend acad. sci.*, **157**: 156 (1913). p 12
J. physiol. path gén, **16**: 204 (1914). p 35
Maynard, L A, *see* Ellis, G
Meerburg, W, *see* Verkade, P E.
Merz, W, *Z. physiol. Chem*, **193** 59 (1930) p 18
Meyerhof, O., *Arch ges. Physiol* (*Pflügers*), **199** 531 (1923). p. 27
Miller, E S. and Burr, G. O., *Proc. Soc. Exptl Biol Med*, **36**: 726 (1937). p. 8
Moore, B and Parker, W. H., *Proc Roy. Soc. London*, **68**: 64 (1901). p 26
Moore, B. and Rockwood, D P, *J Physiol*, **21**: 58 (1897). p 29
Moore, M. G., *see* Anderson, R J.
Moore, T and associates, *Biochem J*, **33**· 1626, 1630, 1625 (1939) p 8
Mühlbock, O and Kaufmann, C, *Biochem Z*, **233** 222 (1931) p 50
Mueller, J H., *J. Biol. Chem.*, **25**. 549 (1916). p 51
Myers, V C and Wardell, E. L, *J Biol Chem*, **36**· 147 (1918) p 51
Neuberg, C and Farber, E, *Biochem Z*, **78**· 238 (1917) p 30
Neuberg, C. and Reinfurth, E., *Biochem. Z*, **89** 365; **92** 234 (1918). p 30
Nishimoto, U, *see* Suzuki, B
Okey, R., *J. Biol Chem*, **88** 367 (1930) p 50
ibid., **100**· lxxv (1933). p 11
Onizawa, J., *J Biochem* (*Japan*), **10**· 45 (1928). p 49, 51
Osato, S. and Heki, M, *J Biol Chem*, **87**. 511 (1930) p 39
Page, I. H., *see* Kirk, E., Rudy, H
Page, I. H and Rudy, H., *Biochem Z.*, **220** 304 (1930) p 11
Z. physiol Chem., **205** 115 (1932) p 17
Palmer, L S, "Carotinoids and Related Pigments, the Chromolipoids," New York, Chemical Catalog Co., (Reinhold Publishing Corp) 1922 p 7

Palmer, L. S. and Eckles, C. H., *J. Biol. Chem.*, 17: 191 (1914). p. 7
Parker, W. H., *see* Moore, B.
Parnas, J., *Biochem Z.*, 56: 17 (1913). p. 17
Paul, S., *see* Hilditch, T. P.
Piontkowski, F A, *see* Caldwell, B. P.
Pottevin, H *Compt. rend acad sci.*, 136: 1152 (1903). p. 11
Powick, W. C, *J Agr. Research*, 26: 323 (1923). p. 10
ibid., 31: 1017 (1925). p. 9
Pregl, F., "Quantitative Organic Analysis," trans. by E. Fylemann, Philadelphia, P. Blakiston's Son and Co. Inc., 1924. p. 47
Price, C. W., *Biochem J.*, 27: 1789 (1933). p. 36
Rae, J. J., *Biochem. J.*, 28: 152 (1934). p. 31
Ralls, J. O., *J. Am. Chem. Soc.*, 55: 2083 (1933). p. 35
Raper, H. S., *J. Biol. Chem*, 14: 117 (1913). p. 29
See also Leathes, J. B
Raspe, F., *see* Erdmann, E.
Réale, L., *see* Artom, C.
Reinfurth, E., *see* Neuberg, C.
Reinhold, J. G, *Am J. Med. Sci.*, 189: 302 (1935). p. 48
Renfrew, A. G., *see* Anderson, R J.
Ridout, J. H., *see* Best, C H.
Rockwood, D P, *see* Moore, B
Rolf, I. P, *see* Levene, P A
Rosenbloom, J and Gies, W J, *Biochem Bull.*, 1: 51 (1912). p 1
Rosenfeld, G, *Biochem Z.*, 200: 280 (1928). p. 38
Rubow, V, *Arch exptl Path. Pharmakol.*, 52: 173 (1905). p. 12
Rudy, H., *see* Page, I H
Rudy, H and Page, I H., *Z physiol Chem.*, 193: 251 (1930). p. 16
Sabatier, P., "Catalysis in Organic Chemistry," trans. by E. E. Reid, New York, D Van Nostrand Co., 1923 p 27
Sabetay, S., *see* Bergmann, M
Salomon, H, *see* Karrer, P.
Sandin, R. B., *see* Wooley, D. W.
Schacht, P., *see* Grün, A
Schäfer, P *see* Magistris, H.
Schaeffer, G., *see* Mayer, A.
Schick, B, *see* Sperry, W. M.
Schmitz, E. and Koch, F., *Biochem. Z.*, 223: 257 (1930). p. 42
Schoenheimer, R, *Z. physiol. Chem*, 180: 1 (1929). p 33
Z. klin Med., 123: 749 (1933) p. 34
Schoenheimer, R. and Dam, H, *Z. physiol Chem*, 215: 59 (1933). p 50
Schoenheimer, R. and Sperry, W M., *J. Biol. Chem.*, 106: 745 (1934). p. 48, 51
Shrewsbury, C. L and Kraybill, H. R., *J. Biol. Chem.*, 101: 701 (1933). p. 7
Simms, H. S, *see* Levene, P. A.
Sinclair, R G., *J. Biol. Chem.*, 86: 579 (1930). p. 14
ibid., 97: xxxiv (1932). p. 14
ibid., 111: 515 (1935). p 21
ibid., 115: 211 (1936). p 43
Skellon, J. H., *J. Soc. Chem. Ind.*, 50: 382T (1931). p. 27
Smedley-MacLean, I., *see* MacLean, H.
Smith, C. S., *J. Cutaneous Diseases*, 36: 436 (1918). p. 6
Smith, J. A B., *see* Channon, H. J.
Smull, J G., *see* Knauss, C. A.
Sobotka, H., "The Chemistry of the Sterids," Baltimore, Williams and Wilkins, 1938. p. 3
Sorber, R. H., *see* Kerr, R H.
Sperry, W. M, *J. Biol Chem.*, 68: 357 (1926). p. 41
See also Schoenheimer, R.
Sperry, W. M. and Bloor, W. R., *J. Biol. Chem*, 60: 261 (1924). p 42
Sperry, W. M. and Schick, B., *Am. J. Diseases Children*, 51: 1372 (1936). p. 34
Steenbock, H., *see* Lease, E. J.
Steenbock, H., Boutwell, P. W. and Kent, H. E, *J. Biol. Chem.*, 41: xii (1920) p. 7
Steger, A., *see* Van Loon, J.
Steuart, D. W, *Analyst*, 48: 155 (1923). p. 31
Stewart, C. P. and White, A. C, *Biochem. J.*, 19: 840 (1925). p. 51
Stoddard, J. L. and Drury, P. E., *J. Biol. Chem.*, 84: 741 (1929). p. 52
Stokoe, W. N., *Biochem. J.*, 22: 80 (1928). p. 10
Strain, W. H., from Gilman, H., "Organic Chemistry, An Advanced Treatise," vol. II, pp. 1220–1398, New York, John Wiley and Sons, Inc., 1938. p. 31, 32
Subbarow, Y., *see* Fiske, C. H.
Süllmann, H. and Versár, F., *Biochem. Z.*, 270: 44 (1934). p 15
Sueyoshi, Y., *J. Biochem. (Japan)*, 13: 145 (1931). p. 15
Sueyoshi, Y. and Furukubo, T., *J. Biochem. (Japan)*, 13: 155 (1931). p 14, 24
Suto, K., *see* Kumagawa, M.
Suzuki, B., *see* Yokoyama, Y.
Suzuki, B. and Inoue, Y., *Proc. Imp Acad. (Tokyo)*, 6: 266 (1930). p. 27
Suzuki, B. and Nishimoto, U., *Proc. Imp. Acad. (Tokyo)*, 6: 262 (1930). p. 31
Suzuki, B. and Yokoyama, Y., *Proc. Imp. Acad. (Tokyo)*, 6: 341 (1930) p. 13
Szent-Györgyi, A. v., *Biochem. Z.*, 136: 112 (1923). p 50
Thannhauser, S. J. and associates, *J. Biol. Chem.*, 129: 709, 717 (1939). p 42
Thannhauser, S. J. and Setz, P., *J. Biol. Chem.*, 116: 533 (1936). p. 49
Thaysen, T. E. H., *Biochem. Z.*, 62: 89 (1914a). p. 11
ibid., 62: 115 (1914b). p. 50
Theimer, E., *see* Grün, A.
Theorell, H., *Biochem. Z.*, 223: 1 (1930). p. 36
Theorell, H. and Widström, G., *Z. ges. exptl. Med.*, 75: 699 (1931). p. 51

Thierfelder, H and Klenk, E, "Die Chemie der Cerebroside und Phosphatide," Berlin, J Springer, 1930. p. 3, 13, 40
Thudichum, J. L W, "Die chemische Konstitution des Gehirns des Menschen, und der Tiere," Tübingen, Pietzcker, 1901 p 17
Tominaga, T., *Biochem Z*. **155** 119 (1925) p 50
Tsujimoto, M, *J. Ind Eng Chem*, **8**: 889 (1916) p 36
J. Chem. Soc., **120**: 78 (1921) p 24
Turner, H. A., *see* Adam, N K
Tuzson, P., *see* Zechmeister, L.
Twitchell, E., *J. Ind Eng Chem*, **6**: 564 (1914); **9** 581 (1917). p. 5
van der Lee, J., *see* Lee, J. van der
Van Loon, J. and Steger, A, *Rec trav. chim.*, **50**. 936 (1931), *C A.*, **25**. 4848 (1931). p 23
Van Slyke, D D., *see* Folch, J, Kirk, E
Van Slyke, D. D. and associates, *Proc. Soc. Exptl Biol. Med*, **32**. 837 (1935) p 42
Venning, E. M., *see* Long, C. N H.
Verkade, P E. and Lee, J. van der, *Biochem J*, **28** 31 (1934). p 26
Verkade, P. E, Lee, J. van der and Meerburg, W., *Rec. trav. chim.*, **51**: 850 (1932). p 10
Verzár, F., *see* Süllmann, H
Verzár, F. and Kúthy, A., *Biochem. Z*. **210**. 265 (1929). p 26
Waldschmidt-Leitz, E., *see* Willstätter, R.
Wardell, E L., *see* Myers, V. C.
Weber, J., *see* Lease, E. J.
Welter, A., *Z. angew. Chem.*, **24**: 385 (1911). p 11
Wentworth, A. H., *see* Folin, O
Wesson, L. G., *J. Biol Chem.*, **65**: 235 (1925). p. 24
West, C. J, *see* Levene, P. A.
Westerkamp, R. F, *see* de Jong, H G B
White, A. C., *see* Stewart, C. P.
Widström, G., *see* Theorell, H.
Williams, M, *see* Gardner, J. A.
Willstatter, R. and Waldschmidt-Leitz, E., *Z. physiol Chem*, **125**: 132 (1923) p 11
Windaus, A, *Z physiol Chem*, **65**. 110 (1910). p 50
ibid., **213**: 147 (1932) p 33
Windaus, A. and Hauth, A., *Ber. deut. chem. Ges*, **39**: 4378 (1906). p. 31
Wooley, D. W. and Sandin, R. B, *J Am. Chem Soc*, **57**: 1078 (1935). p 6
Yasuda, M., *J. Biol Chem.*, **92**. 303 (1931a) p 48, 50
ibid., **94**: 401 (1931b) p. 49
J Biochem (Japan), **25**: 417 (1937). p 35
Yokoyama, Y., *see* Suzuki, B
Yokoyama, Y and Suzuki, B., *Proc Imp. Acad (Tokyo)*, **7**: 12, 226 (1931); **8**. 183, 358 (1932). p. 13
Zechmeister, L, "Carotinoide," Berlin, J. Springer 1934. p 7
Zechmeister, L. and Tuzson, P., *Z physiol. Chem*, **225**: 189 (1934) p 7
Zelinsky, N. D. and Zinzadze, S. R, *Biochem. Z.*, **175**: 335 (1926). p. 38
Zinzadze, S. R., *see* Zelinsky, N. D.

Chapter II

Digestion and Absorption

Of the three great groups of foodstuffs, the fats are important both for their energy value, immediate and reserve, and for their value as raw material from which other lipids are made. This chapter is concerned mainly with a discussion of the voluminous literature on the digestion and absorption of neutral fats (triglycerides of the higher fatty acids). There is included a discussion of other lipids on which, however, relatively little work has been done.

Nutritional Availability of Fats

The neutral fats are mixtures of the triglycerides of the various fatty acids, and these triglycerides may be either simple or mixed as regards their individual fatty acid content. Although the variety of fatty acids found in natural fats is great, only a very few occur widely distributed in large amounts. Of these the most important ones quantitatively are oleic and palmitic, with stearic and linolic (linoleic) next; and others occur in smaller proportions. Because of the limited number of fatty acids involved, it might be expected that fats from various sources would show little differences in their availability as food if they have suitable physical properties; and in general this is found to be the case.

There seems to be no definite relation between the number of carbon atoms in the longer fatty acid chains and their availability as food, according to Ozaki (1926), who investigated the absorption of synthetic fat, although fats with uneven-numbered fatty acids are not as well used. Hydroxy acids are less well used than ordinary fatty acids, and the availability depends on the position of the hydroxyl group and the length of the chain: the longer the chain, the less the utilization. A possible explanation of the difficulty in utilization of the hydroxy acids is that the increase in solubility of the soaps due to the presence of the hydroxyl group would allow an increased concentration of the soap, which in turn would produce increased irritation of the intestine and consequently a laxative effect. It is notable that acetylation of the hydroxyl group in ricinolein greatly increases its nutritive value.

Factors influencing digestion and absorption

Melting point. The melting point of a fat, determined by the nature of its fatty acid components, is the greatest single factor affecting the usefulness of a fat in the animal body. In order to be used to good advantage, a fat must be liquid, or at least soft, at the body temperature of the animal. The need for fluidity shows itself in various ways. Because of its insolubility in water, fat must be emulsified in order to provide surface for effective attack by the water-soluble lipases. For emulsification it must be fluid; fat which is solid at body temperature cannot readily be emulsified in the intestine and may escape digestion. Provided the melting point is low enough, there appears to be very little difference in the percentage availability of food fats, whatever their origin. In fact, they are all so well utilized that it is a question whether, normally, any at all escape absorption, and the small amount of fatty acid present in feces is probably to be regarded as an excretion. An excessive amount of fat in the food may, however, cause waste through its laxative effect.

Direct evidence as to the influence of melting point was given by early experiments of Arnschink (1890), who fed fats of various melting points to dogs and observed the percentage of utilization. His results are given in Table 4.

Table 4. Melting Point and Percentage Utilization (Arnschink, 1890).

	Melting Point (°C.)*	Grams Fed	Percentage Absorbed
Stearin, crystallized	60	20	9
Stearin, melted		20	14
Lard	34	100	97
Mutton fat	49	100	93
Goose fat	25	50	97
Olive oil	0	50	98
Stearin 1 pt., Almond oil 3 pts.	55	20	89

* Body temperature about 37°C.

Significant experimental work on the utilization of fat by the animal body has been carried out by Langworthy and Holmes. In an early series of experiments by these workers (1915), butter and rendered kidney fat of beef, mutton, and pork were used. The subjects were students of 20 to 30 years of age. The averages of digestion according to percentage were: butter fat, 93.9; pork fat, 93.7; beef fat, 88.9; mutton fat, 80.5. Fats of a low melting point were thus better assimilated. A laxative effect was noted if 140 grams of beef fat were eaten in one day.

Similar results were obtained by Holmes (1926), who found that beef fat was digestible according to its melting point; butter and oxtail fat (low melting) 97 per cent utilization; oleo oil, 97 per cent; hard palate, kidney, and marrow fat, 94 per cent; oleostearin (high melting), 84 per

cent. Oxmarrow fat caused disturbances in digestion. Oleomargarine (Holmes, 1925) (3 samples tried on various human subjects) showed a utilization of 97 per cent, 93 per cent, and 97 per cent, in amounts per day of about 80 grams, while 125 grams could be taken without laxative effects.

In experiments on normal human subjects, Langworthy (1923) studied the absorption of 23 animal, 34 vegetable, and 6 hydrogenated fats, by feeding amounts ranging from 50 to 115 grams or more to students or laboratory assistants. The fats, cooked into a pudding with cornstarch, were fed with a simple standard basal ration, and the feces analyzed by standard methods. Results showed no very great differences in the utilization of the fats studied. In the case of hardened (hydrogenated) fats, thoroughness of digestion was approximately inversely proportional to the melting point. Those hydrogenated to a definite melting point and those made by mixing soft and hard fats to produce a fat of this melting point showed no essential differences in the thoroughness of digestion. No laxative effect was observed except in the case of a few fats, *e.g.*, cocoa butter and goose fat.

Irwin, Steenbock and Templin (1936) give a list of fats in the order of their absorption rate with rats as follows: linseed oil most rapidly absorbed, then in order of decreasing rate, olive, whale, soybean, peanut, lard (rancid), cottonseed, cocoa butter, cocoanut, and palm.

McCay and Paul (1938), feeding fat to guinea pigs at the 6 per cent level, found that the fecal lipid was higher after high-melting than after low-melting fats, in contrast to their finding with rats.

Lombroso (1907) found in depancreatized dogs (as in normals) that the fecal fat always has a much higher melting point than the food fat, but this higher melting point is the result of excretion rather than selection. Excreted fat may exceed food fat in these animals.

Iodized fat is absorbed to a considerable extent and the iodine combination persists for some time after reaching the blood and tissues (Artom and Peretti, 1932).

The behavior of tributyrin was examined by Eckstein (1929) and by Davis (1930). Eckstein found that animals could not store tributyrin. Davis found that it was well absorbed by chickens but was quite toxic. In rats, it was absorbed but not stored. On a low carbohydrate diet, it increased acetone body production, but not on a high carbohydrate diet. When tributyrin was injected subcutaneously, the butyryl radical was found in the body fat. Hughes and Wimmer (1935) found that tributyrin when digested with other fats did not appear in the chyle.

Powell (1930) reported that trilaurin was absorbed and produced a depot fat containing as much as 25 per cent lauric acid. Tricaprylin

was deposited only in traces and was apparently changed into one of the ordinary saturated fatty acids since it lowered the iodine number of the stored fat without changing its saponification number.

Nature of the fatty acids. That the melting point may not be the only factor in the digestion of fats is, however, indicated by the *in vitro* experiments of Terroine (1919), who found that there were definite differences in the rate of hydrolysis of various natural fats by the pancreatic secretion of the dog, and that the rate of absorption ran parallel with the rate of hydrolysis. The nature of the fatty acids was apparently of considerable importance. For example, laurel oil was about twice as rapidly saponified by pancreatic juice as coconut oil of about the same melting point. In a study of the action of pancreatic juice on various pure triglycerides of the series from triacetin to tristearin, Terroine (1919) found that the splitting rate increased progressively up to trilaurin, then decreased rapidly. The difference in melting point between stearic (69°C.) and palmitic (63°C.) acids is too small to account for the marked differences in utilization found (stearic 19 per cent, palmitic 63 per cent). Weinstein and Wynne (1936) found that the rate of hydrolysis of fatty acid esters increased with the number of carbon atoms in the acid but decreased with increasing length of the alcohol chain. Peretti (1936) found that the rate of hydrolysis differed with the type of fat used, being high for coconut, olive, poppy, and linseed oils but lower for peanut, sesame, and corn oil.

Soaps are much better utilized than the corresponding fatty acids. Thus Levites (1906) found that stearic acid was 19 per cent, palmitic 63 per cent, and oleic 83 per cent absorbed, while of the corresponding soaps, stearic was 53 per cent, palmitic 61 per cent, and oleic 91 per cent absorbed. The same results were found in dogs with ileocecal fistulas. The sodium soap was almost 2½ times better absorbed than the fatty acid. Solubility in water was undoubtedly an important factor in this case.

Temperature. The effect of temperature on the rate of splitting of glycerides has been investigated by Balls, Matlack and Tucker (1937). Glycerides were emulsified in glycerol and dried bile, buffered to pH 7.5-8.2, and calcium chloride added to precipitate the liberated acids. Splitting of fats containing C_8 or higher acids is greatly dependent on the temperature; the higher the carbon number, the higher the temperature needed. Below C_8, the splitting is little faster at 40°C. than at 20°C. Even at 0°C., the splitting is rapid, while in the case of the higher acids it is nil. In general, as has been reported several times before, the C_7-C_{10} glycerides hydrolyze fastest. Oleic acid behaves in this respect like a C_9 acid.

Vitamins. Mottram, Cramer and Drew (1922) found that the presence of vitamins, especially vitamin B, had a profound influence on the passage of fat through the intestinal wall. With vitamins, fat is absorbed rapidly as a stream of fine droplets running through the cells and a large number of fat droplets can be seen in the central core of the villus. In the absence of vitamins, the fine droplets were not found but only single large drops. Steenbock and associates (1935) found that retardation of fat absorption is not a specific effect of vitamin deficiency but is due to a general slowing down of bodily activities.

Species variation. All animals do not utilize fat equally well. Those which ordinarily do not have much in their diet have difficulty in handling large amounts. McClure and Carr (1925) found with mature pigeons that cod liver oil, cottonseed oil, and mixtures of oleic acid and glycerin, in doses of 5 cc. daily, caused death, and cocoa butter produced digestive disturbances. Fat digestion and absorption in fowls have been investigated by Güntherberg (1930), who found that they can digest and absorb fat well. The fat of maize was best utilized, then that of oats, barley, and wheat, in decreasing order. In normal rabbits, which are unaccustomed to fat, it was not possible to produce alimentary hyperlipemia by feeding fat, probably indicating a low absorption rate. After a period of a month or two on a fat diet (sunflower seeds) visible lipemia was, however, quite regularly found in experiments with rabbits by Horiuchi (1920).

As to the amount of fat which can be absorbed per day by an animal accustomed to it, Arnschink (1890) found for dogs, 346 grams, and humans, 300 grams. Others agree that the maximum for man is about that amount, since feeding 351 grams as bacon and butter caused the appearance of 45 grams in the stool. Rubner (1879) reported the following results for man: in two experiments with bacon, 198 grams were fed and 83 per cent absorbed; 200 grams fed, 92 per cent absorbed; with butter, 240 grams fed, 97 per cent absorbed, 351 grams fed, 87 per cent absorbed. Large amounts of fat in a single dose produce nausea in most individuals.

Von Pettenkofer and Voit (1873) found that after long fat-feeding the fat is no longer as well used as at first. In a 58-day series of feedings on a 32-kilo dog, using 500 grams of meat and 200 grams of fat daily, the feces, toward the end of the period, contained as high as 38 per cent of fat, which at the beginning had been well utilized (98 per cent).

Lipid-splitting Enzymes

The enzymes which bring about hydrolysis of the fatty substances are apparently of two kinds: those which readily hydrolyze the true fats

(neutral fats or triglycerides of the higher fatty acids) but act only slowly on the fatty acid esters of simple alcohols and on the glycerol esters of the lower fatty acids, such as ethyl butyrate and mono- and tributyrin; and those which hydrolyze the true fats slowly or not at all and readily split the simpler water-soluble esters and the phospholipids. According to present usage, members of the first group are generally called "lipases" or "fat-splitters"; those in the second group have been given the name "esterases" by various workers, a name which seems to be suitable. Whether there are actually two classes of enzymes or whether, as Willstätter believed, the two are the same (their difference in behavior being due to the impurities) must be left for later work to decide. They seem at present, however, to be sufficiently distinct in their activity to warrant the use of two terms. Owing to the fact that each of these terms has been used as a general name to cover both groups, there is much confusion in the literature as to the distribution and function of the two groups, a confusion which is not lessened by the fact that their fields of activity overlap to some extent, especially in plants. Wherever possible we have distinguished between the two terms; when it has not been possible to make a distinction the term "lipase" has been used.

In animals, the lipases are apparently confined to the gastrointestinal tract and the pancreas. The esterases have a wide distribution in blood and tissues and seemingly a wide variety of functions. Porter (1916) found enzymes capable of splitting fats and simple esters in many animal fluids and tissues, but generally in minute amounts. Butyrinase was very widely distributed and the most active of these enzymes. Lecithinase was also widely distributed.

One of the most characteristic esterases is that of the liver, while the most important lipase comes from the pancreas; a brief review of the work done with these tissues will indicate the general properties of both types of enzyme and reveal some of the difficulties in distinguishing between them. Terroine (1919) found liver esterase to be active on triglycerides up to caprylic acid but not beyond, and that it readily split aromatic esters which the pancreatic lipase attacked only slowly. Willstätter and Memmen (1924b) found the liver enzyme inactive toward ordinary fats but that it attacked the esters of the lower acids readily. Willstätter (1924) found that pancreatic lipase hydrolyzed fats ten to twenty times as fast as did the esterase from liver. Earlier work by Loevenhart (1906) showed that fresh extracts of pig liver or suspensions of pig liver powder decomposed ethyl butyrate about three times as fast as the corresponding pancreas preparations, whereas the action on olive oil was only about one-seventh that of pig pancreas. On the other hand,

heating dry pancreas at 110°C. destroyed the activity toward butyrate more than that toward olive oil. Loevenhart and Pierce (1907) found that sodium fluoride inhibited the butyrate activity of pancreas extract from 100 to 1000 times as much as its olive oil activity, and inhibited its action on ethyl acetate more than on butyrate, and they concluded that the fatty acid involved was an important, if not the principal, factor. Loevenhart and Souder (1907) reported that bile salts, lecithin, and bile greatly accelerated the action of the pancreatic juice on all esters including oils. The optimum concentration for lower esters was 0.1 per cent bile salts; for olive oil, 2.25 per cent. Loevenhart found further that the following esters of fatty acids were readily hydrolyzed by pancreatic juice: ethyl esters of fatty acids, methyl esters of caprylic acid, and glycerol esters of all the fatty acids. In general, the higher the molecular weight of fatty acids, the easier was the splitting by pancreatic lipase. The following esters were not split by pancreatic juice: methyl salicylate; salts of acid esters of dibasic acids, as, for example, sodium ethyl succinate (therefore iodized substances are probably not split); and esters of aromatic acids (benzoic, salicylate, phenetol).

Loevenhart's work indicated that though liver contained mostly esterase and pancreas mostly lipase, it was possible that liver also contained some lipase and pancreas some esterase. The more recent work of Wolvekamp and Griffioen (1934) corroborates this finding with respect to the pancreas. They reported two varieties of enzyme in the pancreas which could be demonstrated as separate entities by their differences in sensitivity toward elevated temperature and unfavorable pH. They concluded that the differences between the two reside in their colloidal carrier rather than in the active grouping. It is suggested that there may be several esterases in the pancreas, each specific for a definite group of esters. A discussion of the enzymes of liver and pancreas by Sobotka and Glick (1934b) leads them to similar conclusions as to the effects of various factors on the behavior of ester-splitting enzymes.

An earlier study of the lipases (in which they included esterases) by Willstätter and Memmen (1924b) gives a good picture of the difficulties attending work with these enzymes. They found that, unlike the sugar-splitting enzymes, the fat-splitting enzymes are extremely subject to the influences of the conditions of the reaction, the colloidal states, the accompanying substances, and the resulting products of saponification. The activity of pancreatic lipase seems to be dependent upon these accessory circumstances to an extent defying analysis. This difficulty is overcome by methods involving such extensive activation or retardation that the secondary influences become negligible. The enzyme is not measured in

the state in which it occurs but rather in a system of maximum activity arranged by the addition of calcium ion, oleic acid salts, and albumin. In this way the retarding influences of any accompanying substances are excluded. It was found expedient to begin the hydrolysis in alkaline solution and to finish in acid solution. Constancy of pH is thus unnecessary. The substances (calcium oleate, for instance) whose addition activates pancreatic lipase in splitting the various fats and esters, exert a retarding influence upon hepatic lipase. The conditions for analysis of every lipase must therefore be worked out separately. As an example of the difference between the two enzyme activities, they give the following. If dried pancreas is to be replaced by dried liver, there are necessary:

for the hydrolysis of methyl butyrate	4 mg.
" " " " tributyrin	1,000 mg.
" " " " olive oil	100,000 mg.

The difference between lipases and esterases is therefore best shown by their behavior toward different types of fatty acid esters, mainly in regard to length of chain of the acid. Animal esterases work best on simple esters of the lower fatty acids and are less effective as the fatty acid chain lengthens. Lipases work best on the esters of the longer-chain fatty acids, characteristically the fats, and they are much less active on substances such as ethyl butyrate or tributyrin.

Sobotka and Glick (1934a,b), as the result of their work with liver and pancreas enzymes, found that they differ in: (1) physiological function, (2) nature of the protein with which they are found, (3) substrate specificity—the pancreas enzyme for fats, the liver enzyme for simple esters (4) type of kinetics, (5) different affinity for a given substrate, and (6) influence of foreign substances. They follow similar pH curves. Weber and King (1935) conclude from a study of their properties that esterase is an albumin and lipase a globulin. The inhibition of liver esterase by salts of the fatty acids increases with increasing chain length of the fatty acids up to sodium laurate, then decreases to zero for palmitate and stearate. The decrease corresponds to the formation of colloidal aggregates (micelles) with decreasing power of surface tension. The inhibitory effect of unsaturated acids was much greater than that of the saturated (shorter chain?). Kelsey (1939a,b) found enzymes capable of splitting both ordinary fats and cholesterol esters in both pancreas and castor bean and was able to separate them, or at least to separate their activities. He found (1939b) that both esterase and lipase of pancreas were globulins and hence could not be separated, but that the lipase activity could be destroyed by dilute ammonia.

Lipases (Fat-splitting Enzymes)

Gastric lipase

The earliest statement regarding digestion of fat in the stomach is that of Magendie (1825), who found changes in fat in the region of the pylorus. A number of later workers (Terroine, 1919) could find no important changes in fat in the stomach, and until the time of Volhard it was believed that this organ had no part in fat digestion. Volhard (1900, 1901) found that the fat of egg yolk was hydrolyzed to the extent of 78 per cent in the human stomach. His results must, however, be discounted because of his methods of determination which of themselves were such as to bring about hydrolysis. However, they were in considerable measure supported by work of others (Zinsser, 1906; Heinsheimer, 1906; London, 1906) who found splitting of the fat of egg yolk up to 25 per cent. On the other hand, the objection was raised that the observed hydrolysis was not due to a special gastric lipase but to intestinal secretions, including pancreatic lipase, which were known to regurgitate into the stomach when there was much fat present there (Contejean, 1894; Boldyreff, 1904). Significant of what was to come later was the finding of Levites (1906) that gastric hydrolysis of fat is much affected by the pH of the medium, being much more pronounced when it is alkaline; and that of Zinsser (1906), who found that human gastric lipase is very sensitive to pepsin hydrochloride.

Experiments to exclude the possible effect of intestinal juices were soon available. Ogata (1881) closed the pyloric opening with a rubber plug and obtained significant hydrolysis of fat, but it was not certain that communication with the intestine had been entirely prevented. Klemperer and Scheurlen (1889), in anesthetized animals, isolated the stomach by ligatures and obtained no significant digestion. The material on which most of these experiments had been made, egg yolk, was shown by Boldyreff (1911) to be probably unsuitable, since, when mixed with water and kept at body temperature, it developed acidity of about the extent found when it was put in the stomach. The possible presence of a fat-splitting or lecithin-splitting enzyme in egg yolk had been suggested by the work of Wohlgemuth (1905).

This earlier work on gastric lipase may then be summed up by saying that beyond indicating the possibility of the presence of a gastric lipase, nothing definite had been shown. Attack on the problem was continued in various ways without marked progress, although little by little the conditions attending the action of the gastric lipase were worked out. Kastle and Loevenhart (1900) showed that the slight action of extract of gastric mucosa on ethyl butyrate was stopped when the medium was made

acid with hydrochloric acid. Similar results were obtained by Bénech and Guyot (1903) on monobutyrin, but it is questionable whether results obtained with simple water-soluble esters such as these could be applied to the fats. Hull and Keeton (1917) experimented with gastric secretion obtained from dogs by gastric fistulas, by Pavlov accessory stomachs, and by blocking the pylorus. Free flow of secretion was induced by the hormone gastrin injected intramuscularly, and suitable precautions were taken to exclude intestinal secretions in all cases. The presence of lipase was shown by the action of the juice on either ethyl butyrate or neutral olive oil. Controls were run on boiled juice, and the extent of the splitting off of actual fatty acid was shown on the material extracted with ether. Hull and Keeton (1917) found that the most important factor in obtaining a lipolytic gastric juice was the sensitivity of the lipase to acids, and that, when the secretion was neutral or when it was neutralized at once after secretion, either by alkali or protein, lipolysis could be demonstrated. They found that the concentration of the lipase in gastric secretion was five or six times that in the succus entericus or blood serum. Using the Volhard (1901) technic they obtained a fat splitting of 28.2 per cent, and using the Stade (1903) modification, 22.0 per cent. They therefore favored the view that the lipase is a true secretory product of the stomach and that it is capable of doing considerable fat splitting before it is destroyed by the gastric acidity, being comparable in this respect with the behavior in the stomach of the ptyalin of the saliva. The work of Hull and Keeton thus supplies a reasonable explanation for the contradictory results obtained by earlier workers, who had not appreciated the effect of gastric acidity.

Authoritative work on this topic was done by Willstätter and Memmen (1924a) who leave little doubt as to the presence of a gastric lipase. They found that the lipase was secreted in the active form and not as zymogen, that there was a greater yield from the cardiac than from the fundic end of the stomach, and that the glycerol extract of gastric mucosa, while active, was 40 to 600 times weaker than a similar extract of dried pancreas. No certain qualitative difference could be found between it and pancreatic lipase. Its optimum pH was on the alkaline side of neutrality and its action was accelerated by sodium oleate.

Pancreatic lipase

The most important of the intestinal lipases is undoubtedly that supplied by the pancreas, and the first worker to make progress in the study of the pancreatic lipase was Claude Bernard (1856), who found that the pancreatic juice both emulsified and hydrolyzed the fats. The properties of the lipase have been studied mainly through the agency of fistulas,

either natural or artificial. Human pancreatic lipase has been studied by Herter (1880), Glaessner (1904), Bradley (1909) and Wohlgemuth (1912), and that of dogs by Pavlov (1910) and associates. The effect of secretin in bringing about pancreatic secretion, discovered by Bayliss and Starling (1904), has been of great help in collecting the secretion. The normal excitants for the secretion of pancreatic juice are acids (H^+), fats, and water; alkalies have a retarding action. Acids act probably by the formation of secretin rather than by reflex action on the intestine as Pavlov believed, although stimulation of the nerve supply will cause secretion. Fats are found to act as excitants only when partially saponified, and soap or fatty acid is probably therefore the active substance. By the time it reaches the intestine, food fat normally contains enough free fatty acid to form a considerable amount of soap when mixed with the alkali of the intestinal secretions. Water acts for the most part indirectly by stimulating acid gastric secretion. The nervous system undoubtedly also plays an important part in pancreatic secretion, not only as a regulator but also in the production of the secretion (Bylina, 1911). The mechanisms of secretion into the gastrointestinal tract have been excellently reviewed by Ivy (1930). He finds that unsplit fat acts as a secretory stimulant mainly on bile secretion.

The amount of pancreatic juice secreted in a 24-hour period has been found to vary greatly, the average from normal dogs, obtained by pancreatic fistula, being about 22 cc. per kilo per 24 hours. For human beings, the amount is about 600 cc. per day. In the pancreas the amount of extractable lipase is least six hours after a meal, and greatest in fasting, in agreement with which is the statement of Morel and Terroine (1909) that the amount in the secreted juice falls off as the secretion proceeds. The relative lipase activity of pig, beef, and sheep pancreas was found by Chrzazcz and Janicki (1938) to be in a ratio of 17:11:12, taking into account the relative size and consistency of the glands in the different animals.

The pancreatic lipase (steapsin) hydrolyzes the fats to fatty acids and glycerol, an action which is reversible, as was first reported by Pottevin (1903). By using oleic acid homogenized with glycerol and mixed with glycerol extract of pancreas (therefore with excess of glycerol) he was able to get a combination of about 33 per cent of the oleic acid in 50 hours at 38°C. Artom and Réale (1936), using fat-free pancreas powder, obtained over 50 per cent esterification. They reported that the secondary alcohol group of the glycerol was difficult to esterify. Armstrong and Gosney (1914) made an exact study of the synthesis by castor bean lipase. They found that, proceeding in either direction with the glyceride or with glycerol and oleic acid in the proportions found in the natural

glyceride, the equilibrium point was reached when about 40 per cent of the acid was combined. Apparently during synthesis the compounds formed were mainly diglycerides. Near the beginning of hydrolysis with excess of water a small amount of a lower (mono-) glyceride was present, but as the action continued the molecule was completely hydrolyzed. When only a small proportion of water was present a greater proportion of mono- and diglycerides was produced. Conversely, when synthesis was effected in the presence of water more of the triglyceride was formed. Synthesis in the presence of extra glycerol resulted, as would be expected in a proportionately greater combination of fatty acids, with the formation of more of the lower types of glyceride, although the diglyceride was probably still the main product.

Pancreatic lipase is secreted chiefly in the active form, but its activity is greatly influenced by foreign substances. Its action is accelerated by bile (bile salts) and by many other substances, as for example, blood serum, soaps, saponins, alcohol, etc.; it is inhibited by cholesterol. Rosenheim (1910) has succeeded in separating from the lipase of pancreatic extracts (in glycerol) a co-enzyme without which the enzyme is inactive. As is often the case with co-enzymes, this one is heat-stable. Since the inactive enzyme is activated by blood serum, the assumption is made that the activating substance is a hormone produced by the pancreas and secreted into the blood.

Preparation. The pancreatic lipase, although secreted with the pancreatic juice in water-soluble form, is with difficulty extracted from the gland by water, glycerol being generally used for the purpose; the active extract usually contains the enzyme, not in solution but in suspension, since, if the muddy suspension is cleared by filtration, the filtrate has little or no lipolytic activity.

The best method for the preparation of pancreatic lipase is probably that of Willstätter and Memmen (1924b), which is as follows: Pancreas was dried with acetone and ether, extracted with water or 87 per cent glycerol (preferably glycerol because the lipase is unstable in water), centrifuged strongly, diluted in water, and allowed to settle and clear. The lipase was adsorbed on aluminum hydroxide or kaolin, dissolved with ammonium phosphate and the phosphate removed with magnesia mixture. The adsorption was repeated and the enzyme finally adsorbed on tristearin or cholesterol. It was recovered with ammonium phosphate or dried in vacuum, the residue extracted with benzene, and finally washed with alcohol and ether. By these means it may be concentrated about three hundred times.

Properties. Pancreatic lipase, although secreted as a water solution, is unstable in water but may be preserved with glycerol. It gives a weak

protein reaction but no carbohydrate reaction. It contains much ash and about 10 per cent nitrogen. The optimum temperature for its activity is about 40°C., but it is slightly active at 0°C. Its activity is weakened by ten minutes' heating at 45°C. and destroyed at 55°C. Pancreatic lipase hydrolyzes all glycerol esters including the hydrins. Triglycerides are most readily hydrolyzed, diglycerides next, and monoglycerides least readily. Of the natural fats, trilaurin and triolein appear to be the most easily split. The higher the content of triolein, the more readily are the fats split, hydrolyzability appearing, as noted previously, to be dependent to a considerable extent on fluidity at body temperature. As has been mentioned, melting point is not the only reason for the differences. The chemical nature of the fatty acids is important.

Pancreatic juice is undoubtedly of the greatest importance in fat digestion, but it is peculiar in several respects. Complete shutting off of the juice from the intestine does not at once result in diminished fat splitting, but only after a considerable time; and implantation of pancreas under the skin results, according to Jansen (1911), in satisfactory absorption. Nothman and Wendt (1932) found that the absorption of fatty acids is independent of pancreatic secretion. Action of intestinal lipases, if not bacteria, is undoubtedly a factor in explaining these peculiarities. If the pancreas is diseased, the splitting may be complete and yet absorption may be defective.

Intestinal lipase

There is present in the intestine a lipase which does not originate in the pancreas. Schiff (1892) was apparently the first to note the digestion of fat by the intestinal juices, since he found considerable splitting in depancreatized animals. He, however, ascribed it to the alkali. Boldyreff (1904, 1907) collected secretions from a Thiry-Vella fistula and found that it rather feebly hydrolyzed monobutyrin and various neutral fats. Bile greatly aided the action. Kalaboukoff and Terroine (1907) found that a glycerol extract of intestinal mucosa was lipolytic and that the acceleration produced by bile was due to the bile salts.

Plant lipases

Seeds. The plant lipase most widely investigated is that of the castor bean (*Ricinus*), and all workers are agreed that it is very active toward true fats. Green (1890) did the first important work on it and was soon followed by Connstein, Hoyer and Wartenberg (1902). The enzyme is activated by acid, which is ordinarily supplied during the process of germination, but resting seeds are equally active if acid is added. The enzyme works best at a pH of about 5, which is the acidity produced

during sprouting; but, after activation, it may be effective over a considerable pH range. The lipase is insoluble in water (Falk, 1924, p. 137) and in the absence of fat is inactivated by water. Fat preserves its activity. Its optimum temperature is 35°C., and it is destroyed by heat at about 60°C., although when mixed with oil it resists much higher temperatures (up to 160°C.). Armstrong (1905) and his associates found that *Ricinus* lipase, although active toward fats, is very slightly active toward lower esters. With this enzyme, they (Armstrong and Gosney, 1914) were able to demonstrate excellent reversibility of action, using oleic acid and triolein. Equally good results, but with a somewhat different equilibrium point, were obtained by Morel and Velluz (1928), a difference which they ascribed to a greater complexity of the enzyme used by Armstrong. Willstätter and Waldschmidt-Leitz (1924) have carried out important work in the purification and determination of the properties of the castor bean lipase. In contrast to Armstrong, they found that it split fats and simpler esters almost equally well. Falk and Sugiura (1915) claimed to be able to separate the lipase and esterase properties of the castor bean enzymes by reason of the solubility of the esterase in water but not in salt solution, the solubilities of the lipase being just the reverse. Both enzymes were either protein in nature or carried by proteins. Willstätter and Waldschmidt-Leitz (1924) have denied the possibility of the separation into two enzymes. The preparation and properties of a highly active *Ricinus* lipase preparation is described by Longenecker and Haley (1935). They found that their preparation catalyzed the hydrolysis of the following oils listed in order of decreasing percentage hydrolysis in a given time: peanut, castor, corn, cottonseed, soybean, rape, olive, linseed, neatsfoot, peach kernel, coconut, whale, fish, and sperm oils. The preparation showed no specificity in its attack on glyceride molecules containing carbon chains of different lengths. Kelsey (1939c) has reported that *Ricinus* lipase does not act on the cholesterol esters of blood.

Lipases have been found in various other seeds. Traetta-Mosca and Milletti (1923) found a lipase in sunflower seeds (*Helianthus annus*), and Bournot (1913, 1914) in *Chelidonium majus*. A lipase was found in the papaw (*Carica papaya*) by Sandberg and Brand (1925) which had the following properties: insoluble in water, optimum temperature 35-40°C., optimum pH 5.8-6.2 in acetate buffer, activated by calcium chloride. It differed from castor bean lipase in optimum pH and in activation by calcium chloride.

Lower organisms. Lipases are found in various lower organisms: molds (*Aspergillus*) (Schenker, 1921), yeasts (Smedley-McLean, 1922), and bacteria (Jensen and Grettie, 1937; Vickery, 1936). Gorbach and

Günter (1932) found a lipase in yeast with an optimum reaction of pH 6.0 to 6.8, optimum temperature 30°C.

Schenker (1921) reports results of a study of the action of *Aspergillus niger* on various fats, in which the determination of the lipase activity was made by titration with potassium hydroxide of the fatty acids produced. It was shown that the lipase activity of the plant enabled it to utilize the carbon of fats for its own carbon need when no other source was present. The temperature curves for growth and lipase activity were nearly parallel. The pH of the substrate played an important part in the process, weak acidity favoring the fat splitting, while alkalinity retarded it. Although *Aspergillus* could and did form lipase on many kinds of substrates, it appeared that a medium which contained fat was particularly stimulating to lipase formation. Sucrose and other sugars apparently retarded lipase formation, but nitrogenous compounds, such as peptone, had no effect. Oxalic acid was found to occur as the result of the growth of *Aspegillus*, whether fat was present or not. The enzyme split mono- and tributyrin easily at the temperature of 40°C. and was destroyed by dry heat. These enzymes apparently are more widely active than animal lipase.

The hydrolytic activity of bacteria (Michaelis and Nakahara, 1923) on tributyrin has been studied by using the stalagmometric method. Fat-splitting activity among the pathogenic bacteria was noted in the case of the pyogenic cocci and the tubercle bacillus, but not in that of the diphtheria bacillus, pneumococcus, bacilli of the colon group, or hay bacillus. A saprophyte with marked lipolytic power was most active in a range of pH from 7.2 to 9.0, and the lipase of yeast was most active between 5.8 and 6.2.

Methods of measuring activity

A procedure suitable for testing lipase activity was devised by Palmer (1922). The material to be tested was added in the form of an extract or finely minced paste to at least 75 cc. of artificial "milk", prepared by grinding a suitable oil in hydrated acacia and diluting the emulsion with water. Formaldehyde (1:1500) was added to the "milk" as preservative. The initial acidity was determined by withdrawing a 25-cc. aliquot and adding it to 100 cc. of acetone-ether (3:1) and titrating with 0.1*N* alcoholic potassium hydroxide solution, using phenolphthalein as indicator. The remainder of the "milk" was incubated with the enzyme preparation for twenty-four hours at 38°C., with occasional rotation of the flasks, and the titration repeated on another 25-cc. aliquot. The features of the method are: (1) the use of an artificial "milk" containing no acid-pro-

ducing substance other than the emulsified oil; (2) the determination of the acidity on aliquot portions of the emulsion.

Various other methods for testing hydrolysis have been used: Hanriot and Camus (1897) used monobutyrin as a substrate and titrated the butyric acid set free. Stade (1903) extracted the ferment mixture with ether and determined the free fatty acids in the extract, thus eliminating the effect of non-fat acids produced in the ferment mixture. Rona and Michaelis (1911) determined the splitting by the change in surface tension as shown by the drop method. Rona and Lasnitzki (1924) determined the esterase effect of blood serum on tributyrin by measuring the carbon dioxide evolved from bicarbonate.

Some of these methods have been criticized. The danger in drawing inferences from measurements by titrations in solutions containing protein has been pointed out by Amaki (1924), who showed that errors may be introduced by the split products of protein when the titrations are performed directly on the digestion mixtures. By isolating (by extraction) the free fatty acids produced by the lipolytic enzymes, he found that blood serum esterase was inactive toward neutral fat, as were also organ extracts except that of the pancreas. The former idea that lung or liver held an important place in fat metabolism, he believed, rested on this error in measurement. Comments on measurement of lipase action are made by Wwedensky and Dobrowitzky (1927).

Esterases (Ester-splitting Enzymes)

Origin

There are probably several esterases in blood and also in tissues, since the so-called lecithinases, cholesterol-esterases, etc., would fall in this class. In most of the earlier work no distinction is made between various esterases, or for that matter between esterases and lipases, and it is not yet possible to present a clear picture of these substances and their behavior. The blood esterases apparently do not originate in the pancreas, since the amount in the blood does not change upon removal of the pancreas (v. Hess, 1911). Their possible origin in the liver is indicated by the work of Jobling, Eggstein and Petersen (1915), who found an increase after phosphorus and chloroform poisoning while the liver esterase diminished, and of Prewitt (1923), who found that the liver enzyme could be washed out by perfusion with blood.

On the other hand, blood lipase, when it occurs, originates in the pancreas. Caro (1920) came to this conclusion after finding no relation between the serum lipase and the lymphocyte count. In confirmation of this supposition is the work of Hiruma (1923), who reported that ligation of the pancreatic duct increased the lipase content of the blood. He came

to the conclusion that the blood lipase originated in the pancreas, passed into the blood, and was taken up by the organs, a finding confirmed by Cherry and Crandall (1932), who also found that under these conditions there was no significant change in the esterase content.

Occurrence

Although all tissues undoubtedly contain lipid-splitting enzymes, at least in small amounts, those of the blood are probably the most important in the intermediary metabolism of the lipids. The sum of the evidence at the present time is against the occurrence of a true lipase or fat-splitting enzyme in normal blood, although lipase may appear in the blood under unusual conditions. Hanriot (1898) concluded that the enzyme present in blood was a true lipase. Arthus (1902) found, on the contrary, that it was an esterase distinct from the true lipases; as did also Noguchi (1907), who found that the blood enzyme did not split the higher glycerides, and that when pancreatic lipase by accident or in pathological conditions entered the blood, there was danger of lipolytic hemolysis. Esterase and lipase in blood under different conditions have been studied by Crandall and Cherry (1931), who used ethyl butyrate and an acacia emulsion of olive oil as substrates. Normally, they found no lipase in the blood of the animals studied, but it was made to appear: (a) by ligation of the pancreatic ducts, and (b) by removal of portions of pancreas, with a maximum at about 24 hours. Esterase was not changed. Eck fistula in dogs resulted in lipase in the blood in most cases after two or three weeks. Obstructive jaundice resulted in prompt increase of blood lipase. Lipase was normally found only in intestine, pancreas, liver, and spleen. In the disease, multiple sclerosis, they (1932) found an enzyme capable of splitting olive oil in 78 per cent of their cases, and also in 80 per cent of cases of liver disease and 8 per cent of other diseases, while the blood esterase showed no significant change.

Blood esterase was found by Greene and Summers (1916) to be increased by a fat meal. The effect was marked, double or more, after a long fast, but feeding normal puppies caused only a slight increase in blood esterase. On the other hand, Bach (1922) found that feeding, even with a high-fat diet, did not influence the serum lipase. Tsuji (1932) reported that the esterase content of the blood was decreased by adrenalin, painful stimuli, choline, or hemorrhage. He found the esterase content of lymph to be less than that of blood. Reed (1923) found in lymphocytes no enzyme capable of splitting fat or tubercle wax.

Enzymes capable of splitting simple esters are widely distributed in animal organs and tissues. Mayer and Morel (1919) found that lung tissue contained an enzyme which hydrolyzed various low-molecular esters

at about the same rate as that found by Morel and Terroine (1912) for inactivated pancreatice extract, but which differed from pancreatic lipase because bile salts did not accelerate its activity. In studying homologous series of fatty acid esters, definite maxima in ease of splitting were found. This enzyme attacked triolein very slowly.

Much work on the tissue esterases has been done by Falk (1924, p. 178) and his associates, using normal and carcinomatous rat tissues with various simple esters as substrates. The carcinoma esterase has only slight action on all esters. The esterases of muscle (heart and leg) and brain also were only slightly active and, in general, the activity was greatest for kidney and liver, then testes, spleen, and lungs. Of the various animal tissues tried, rat tissues were most active, followed by rabbit, beef, and human tissues, in the order named. Brain extracts showed the smallest absolute activity in every case, and the same picture of relative activity was observed for every animal studied. The absolute values of the activity of tumors of human origin varied considerably among themselves; but, in general, they were found to be small as compared with most normal tissues and were more nearly of the order of magnitude of those found in muscle or brain extracts than in other tissues. Falk and McGuire (1935) found that kidney extracts hydrolyze ethyl esters 50-100 per cent faster than methyl esters; but with the lung esterase the activity was the same. Liver extracts hydrolyzed ethyl and methyl esters of butyric and benzoic acids at the same rate, but were more active on ethyl than on methyl acetates.

An interesting series of investigations on the behavior of liver esterase toward dicarboxylic esters has been carried out by Lewis and his associates (1923, 1926). They found, in confirmation of earlier work, that hydrolysis of the diethyl esters of succinic and malonic acids by the esterase of hog liver proceeded rapidly to an equilibrium which corresponded to the removal of one ethyl group from the diethyl ester. In the hydrolysis of the diethyl esters of adipic and glutaric acids under like conditions, equilibrium was reached when the cleavage corresponding to the removal of both the ethyl groups was nearly complete. The hydrolysis of these esters followed a course similar to that observed with the simple hydrolysis of diethyladipate by liver esterase. In experiments in which the hydrolysis of the diethyl esters of malonic, succinic, glutaric, and adipic acids was studied, the extent of hydrolysis increased with increasing molecular weight of the acids. Uzawa (1932) prepared a mono- and also a di-esterase from Taka and rice hulls.

Kernot and Hills (1932) made a study of the tissue esterase of carp, finding it present in all tissues but most abundant in the liver. It showed the same preferential splitting of *l*-tartaric acid esters over the *d*-form,

as was shown by beef liver esterase. Enzyme from pig's liver (Willstätter and Memmen, 1924b) produced *d*-mandelic acid from *d*-*l*-mandelic ester, whereas that from pancreas yielded the *l*-isomer.

Cholesterol esterases seem to be fairly widely distributed. Abderhalden (1911) and Thannhauser (1923) found that the pancreatic juice and intestinal secretions split cholesterol esters. Kondo (1910) and Schultz (1912) found cholesterol esterases in blood corpuscles (not in plasma), and in liver. Since he found no change in the relation of free and bound cholesterol on autolysis, Mueller (1916a,b) questioned these results. Porter (1916) reported a cholesterol esterase in the blood. Nomura (1924) reported cholesterolase in different organs and tissues of various animals, but not in blood. Shope (1928) found that the cholesterol ester of blood diminished after death, because of the action of cholesterol esterase. He found cholesterol esterase present in many animal tissues. Sperry (1935) has established the presence in normal human blood serum of an esterase which on incubation brings about a marked esterification of free cholesterol. The optimum was at pH 8.0. The effect was abolished by heating the serum to 55-60°C., and there was no esterification of the free cholesterol of corpuscles. Tissue extracts inhibited the reaction to some extent. Bile salts inhibited the esterification and, in sufficient concentration, stopped it entirely. In dog serum, addition of bile salts caused a reversal of the reaction with hydrolysis more or less in proportion to the concentration of the bile salt until, with the larger amounts, complete splitting occurred.

The presence of phospholipid-splitting enzymes has been demonstrated in many tissues. Thiele (1913) and Porter (1916) reported a ferment in blood which could split lecithin but not fat (therefore an esterase and not a lipase). King (1931) found a lecithinase in many tissues. Its optimum reaction was pH 7.5 (as compared with phosphatase, pH 8.9). Its optimum temperature was body temperature. It was stable in neutral solutions, but was destroyed by acid or alkali at 88°C. Later (1934), he further investigated an enzyme found in kidney and intestinal mucosa which slowly hydrolyzes lecithin, liberating phosphoric acid. Hydrolecithin is equally well attacked, cephalin and phosphatidic acid more slowly, while lysolecithin is split about twice as rapidly. The optimum reaction is pH 7.5. Takata (1933) found that cholic acid somewhat increased the splitting of the phosphoric acid from liver and kidney lecithin by the lecithinase from these organs. Yosinaga (1936) described a lecithinase in Takadiastase which liberated choline at pH 4. Belfanti, Contardi and Ercoli (1936) have reviewed the work on some of these enzymes, including lecithinase A and B, cholinephosphatase, and glycerophosphatase.

Function of esterases

The results obtained by the various workers show that esterases (enzymes capable of splitting a wide variety of esters with varying readiness) are of wide distribution, in this respect being similar to another and equally indefinite group, the catalases. All are agreed that these enzymes do not split fats readily and that therefore a distinction from lipases is desirable, but differentiation is difficult because the differences between the two are not clear-cut.

The significance of the esterases in fat metabolism is problematical. Loevenhart's work (1902) seemed to show that they are present in largest amount in those tissues which have to do with fat metabolism, such as the secreting mammary gland and the subcutaneous fat deposits, and he enunciated a theory of fat transportation, with reference particularly to the passage across cell walls, to the effect that the fat can enter or leave cells only after a hydrolysis similar to that known to take place in the intestine, the esterases providing the necessary means of hydrolysis. Bradley (1913a) was unable to corroborate Loevenhart's findings in some important particulars. Using as his test the splitting of actual fats instead of ethyl butyrate as Loevenhart had done, he found that some of the tissues most active in the production of fat, such as the secreting mammary gland, were poorer in true lipase than others which never normally contained or produced much fat, such as the lung, kidney, and muscle. He concluded that the quantitative comparison of the fat and lipase content of animal tissue gave no support to Loevenhart's theory. However, the presence in blood of esterases which are able to hydrolyze both the phospholipids and cholesterol esters has been demonstrated (see p. 76). This relation of esterases to substances which are fatty acid derivatives and probable stages in fatty acid metabolism indicates that the esterases may be important in some stages of fat metabolism, a possibility which is emphasized by the participation of phospholipid in the transport of fatty acids across the intestinal mucosa and in the blood, as shown by the work of Sinclair and of Verzár and associates (see under Fat Absorption).

In the light of what has already been done, it appears that the true lipases are normally confined to the pancreas and alimentary tract, their functions being to prepare the lipids for entry into the organism. After the lipids are once in the blood stream, the work may be taken up by the esterases, which, since they can split the phospholipids and cholesterol esters, can probably also synthesize them. As will be noted later, there appears to be a definite interrelation between fats, phospholipids, and cholesterol esters in metabolism and in life processes in general, and it is quite possible that these esterases have a part in it.

A practical use of blood esterases as a measure of liver function has been made by Whipple (1913) and other workers. The esterase content of the blood was found to increase greatly when the liver was damaged. His method of esterase measurement was by titration of ethyl butyrate-serum preparations after digestion at body temperature. Rona and Lasnitzki (1924) determined the esterase effect of blood serum on tributyrin by measuring the carbon dioxide evolved from bicarbonate.

Fat Digestion

The general rule in digestion appears to be a maximum hydrolysis of the complex food substances consistent with retaining in the fragments the characteristic properties of the original substances. Also the products must be either water-soluble or easily and simply made water-soluble with the help of the materials available. Thus the carbohydrates are digested to monosaccharides and the proteins to amino acids, both of which retain the characteristic properties of the original substances, but are relatively small molecules and are more soluble in water than the original substances. Any further splitting would destroy the special characteristics. Fats are split to fatty acids and glycerol, the fatty acids retaining the characteristics of the fats including their insolubility in water. Any further splitting would alter their characteristic fatty properties. The fatty acids may be made water-soluble by neutralization with the alkali of the intestinal secretions, by the bile, and to some extent by formation of phospholipids; glycerol is water-soluble.

The fats enter the blood stream largely as such and are insoluble in the body fluids. Digestion and absorption of the fats thus presents difficulties not found with the proteins and carbohydrates, and special mechanisms are provided. The first step is that of emulsification: the size of the fat particles is reduced to a point where their surface is great enough for the fat-insoluble lipases to bring about sufficiently rapid hydrolysis. Emulsification in the intestine is effected partly by the soaps formed from the free fatty acids in the food combined with the alkali of the intestinal secretions. The soaps together with bile and protective colloidal substances—mucin, lecithin, etc.—in the secretions, along with the motions of the intestine, bring about emulsification of the fats soon after they enter the intestine. The fact that emulsions are not always found in the intestine during fat absorption (Moore and Rockwood, 1897a, b) may mean only that absorption is rapid. The fact, which is now quite well established, that the reaction of the small intestine is slightly on the acid side of neutrality and that therefore soaps, and consequently soap emulsions, cannot exist there is more difficult to meet. That there

is an emulsification of fats near the point of entry of the bile and pancreatic juice was first noted by Claude Bernard (1856) and Dastre (1890), and has been confirmed many times. The emulsion may not persist long, but probably long enough for hydrolysis of the fats to occur.

Digestion in the Stomach

Factors in fat splitting

The sum of the work to date leaves little doubt that some lipase is secreted by the stomach. Whether much fat splitting takes place there will depend on a number of factors, among which may be mentioned the following. (a) The *acidity* of the stomach contents: high acidity destroys and lower acidity inhibits the activity of the gastric lipase; the acidity is dependent on the amount of acid secreted and on the amount of neutralizing substance (ordinarily protein) present. (b) The *state of division* of the fat: since the lipase and the fat have no common solvent, the splitting can take place only at the surface of the fat particles, and unless the particles are very small and the surface correspondingly great (as in an emulsion) not much splitting is likely. Probably the acidity is rarely weak enough in the stomach to allow the formation of soap emulsions, so that the lipolytic activity is likely to be limited to natural emulsions, such as milk or egg yolk, in which case the splitting might be considerable. (c) The *length of time* the food remains in the stomach: when much fat is present in the food, the emptying of the stomach is slowed and the secretion of gastric juice, both pepsin and acid, is inhibited. Under these conditions, as has been shown by Boldyreff (1908, 1911), the intestinal secretions, including pancreatic juice and bile, pass into the stomach as the result of antiperistaltic movements, and the contents of the stomach may become alkaline, in which case fat digestion undoubtly takes place (Levites, 1906).

While Frank (1923) reported mostly negative results from the effect of fat in food on gastric secretion, other evidence is positive regarding the effects of fat on gastric digestion and secretion. Lockwood and Chamberlin (1923) found that administration of olive oil before meals caused reduction in average gastric acidity and lowering of the high point of acidity. Regurgitation of bile took place in 80 per cent of the cases. Kalk and Dissé (1924) reported that sesame oil administered half an hour before an alcohol test breakfast slightly increased the acidity and reduced the secretion and evacuation time, because of an earlier and increased regurgitation from the duodenum. Margarin prolonged the emptying time, and rancid margarin increased the acidity while reducing the evacuation and secretion time. Boldyreff and Kellogg (1924) experi-

mented with rectal administration of different oils, and found that ten to twelve hours after the introduction of 50 to 100 cc. of olive or linseed oil into the rectum of dogs the volume of gastric juice secreted, after excitation by displaying food or by sham feeding, was much smaller than that in control experiments without oil. The acidity and proteolytic activity of the juice were normal. Neutralized oil produced a similar but less marked and more delayed effect, so that the inhibition was ascribed to the fatty acids. Cod liver oil was less inhibitive than olive or linseed oil. The relatively slight action of butter was ascribed to the fact that it did not melt in the rectum and consequently did not pass through the ileocecal valve into the small intestine. Mineral oil was inactive, a fact which has been confirmed by Smidt (1923) and by Lim, Ivy and McCarthy (1925), who demonstrated also a slight action of fat in the stomach. The mechanism appears to be humoral. Ivy, Lim, and McCarthy (1925) and Kosaka and Lim (1930), experimenting with dogs having Heidenhain pouches, found that fat given by stomach tube inhibited gastric secretion, but bile, either by mouth or intravenously, did not. After removal of the gall bladder, inhibition could still be produced. Ivy's cystokinin was found to inhibit secretion.

If the food is not very fatty, there is little slowing of gastric secretion and no regurgitation; whatever fat digestion there is in the stomach under the circumstances results from the activity of the gastric lipase, and is ordinarily slight unless the fat is emulsified as in milk or egg yolk. Toward the end of gastric digestion the intestinal secretions, including bile, may flow back into the stomach.

It can therefore be stated that under the most favorable conditions there may be considerable digestion in the stomach, but that in most cases gastric digestion of fat is negligible.

Absorption from the stomach

Klemperer and Scheurlen (1889), by ligating the intestine of dogs below the pylorus and weighing fat before and after three to six hours in the stomach, found that none had been absorbed. The objection might be raised in this case, as in many similar ones, that the shock of the operative procedures was responsible for the failure. Histological observations from von Kölliker onward have demonstrated fat droplets in the gastric epithelium, although none were seen in the lymphatics. Weiss (1912) believed that absorption into the epithelium was confined to young animals, in which belief he is opposed by Greene and Skaer (1913), who found absorption (into the epithelium) in both young and old animals and noted also that the amount of absorbed material (ob-

served by staining) and the depth of penetration depended on the length of stay of the fat in the stomach. The histological picture was found by these observers to resemble strongly the appearance of the intestinal mucosa during fat absorption. After the fat left the stomach the cycle reversed and the fat disappeared.

Mendel and Baumann (1915) studied the absorption of fat by the stomach histologically and chemically, and confirmed in general the work of Greene and Skaer, although in some animals they found no penetration. They found no change in the fat content of the blood as a result of the presence of fat in the stomach, but they pointed out that the absorption would necessarily be slow and that the fat may have been removed from the blood as fast as absorbed. That absorption of other substances went on normally in these same animals was shown by tests with iodides. On feeding fat stained with Sudan III no color could be observed in the lymph or in the blood.

Inouye (1924) found that some fat could be absorbed through the stomach, increasing the fat content of the thoracic lymph slightly, but the increase was very small.

Absorption of fat through the stomach is thus seen to be improbable, although the mucous membrane can take up fat in the same way as the mucous membrane of the intestine. This failure to complete the absorption process is in agreement with the general statement that the stomach is not an absorbing organ. The function of the stomach toward fat consists ordinarily in freeing it from enclosing protein membranes by digestion, bringing about a small amount of hydrolysis with the liberation of a little fatty acid to be added to the small amount normally present in good food fat, thus providing material for the formation in the intestine of enough soaps to promote emulsification. Another function of the stomach apparently is to regulate the entry of fat into the intestine and so avoid the flooding of the intestine with a substance relatively difficult to digest.

Intestinal Digestion

Passage from the stomach

Fat in the food tends to delay the emptying of the stomach, and the effect varies with the proportion of fat in the food. When the proportion is large, the rate of passage of the food and hence of the fat into the intestine is decreased, with the result that in all cases the fat is delivered into the intestine in small portions. Non-emulsified fats leave the stomach more slowly than emulsions and, when unmixed with food, they leave at a still slower rate (olive oil and, probably, other liquid fats are excep-

tions; 90 per cent of olive oil is gone in two hours). Von Fejer (1913) found that the rate of departure of non-emulsified fats varied with their melting point and viscosity. The less viscous fats leave the stomach more quickly than the more viscous. Fat mixed with food separates to some extent and may pass out separately.

Thus, in all cases except where the fat is taken in quantity in the form of oil or as emulsions, it is delivered into the intestine in small portions. When it reaches the intestine in large quantities, diarrhoea may be produced, either through the action of the fat itself or, more probably, as the result of irritation produced by the abnormally large amount of soaps formed. When the amount of fat in the food is so large that there is a great inhibition of gastric secretion, the pylorus appears to lose its tone after some hours and allows the passage of intestinal contents (bile and pancreatic secretion with its lipase) into the stomach, and considerable hydrolysis of the fats probably takes place. Boldyreff has shown that this regurgitation may be made to take place readily in humans by feeding fat containing fatty acid.

One result of the normal functioning of the gastric mechanism is therefore the delivery of the fats to the intestine in small portions, which undoubtedly is an important factor in the completeness of its digestion and absorption.

Factors in intestinal digestion

The digestion of fat in the intestine has been fully reviewed so many times that another detailed attempt seems hardly necessary. For a very complete discussion of the factors entering into the digestion and absorption of fat the reader is referred to Terroine (1919) and Leathes and Raper (1925). Normally, the provisions for the digestion of the fats in the intestine are such as to insure practically complete splitting. Fat is delivered to the intestine from the stomach in small amounts; when there is little fat in the food, this follows as a matter of course; when fat is present in large proportion, emptying of the stomach is slowed, effecting the same result. Emulsification is an important factor in the hydrolysis, and there is normally in the duodenum abundant provision for it. Lipase is also abundant, being found both in the pancreatic and in the intestinal secretions. Bile apparently acts as the common solvent for fat and lipase. Under favorable conditions, a considerable quantity of the fat may have been previously split by the gastric lipase, and the amount of lipase in the pancreatic secretion alone is sufficient to digest quickly several times the amount of fat supplied in the ordinary diet. The intestinal lipase can also probably bring about the splitting of the daily quota of fat, since in cases

where the pancreatic secretion is lacking, very little unsplit fat is found in the feces. Added to these factors is the continuous absorption which removes the products of digestion, providing for rapid and complete hydrolysis. Under these conditions, it is probable that the amount of fat which normally escapes hydrolysis is negligibly small.

Enzyme action. The main lipase is that of the pancreatic secretion of which the properties have been discussed under the heading, "Lipases." The amount of pancreatic juice secreted in a day is quite large and contains enough lipase to digest several times the amount of fat ordinarily found in the diet. Although the pancreatic lipase is undoubtedly very important for fat digestion, the organism can get along fairly well without it, the function being apparently taken over by other lipases such as those of the intestine and possibly by bacteria.

Results of exclusion of the pancreatic secretion from the intestine vary with the extent of exclusion. When a small amount of juice can reach the intestine the absorption is practically normal. When the secretion is completely excluded, the reports of absorption vary. Pratt (1916), working with dogs from the intestine of which the pancreatic excretion was completely excluded, found that absorption of protein and fat was poor, although the fat was well hydrolyzed. Feeding of raw pancreas resulted in better absorption. McClure, Vincent and Pratt (1917) found further that dogs with a subcutaneous transplant, which secreted and discharged pancreatic juice externally, absorbed no more fat than dogs in which the pancreatic remnant was undergoing rapid atrophy and sclerosis. Hence, the presence of pancreatic tissue in the body was not found to influence the amount of fat absorbed by the intestine, a finding which is not in agreement with work noted below. Fleckseder (1908) found absorption still quite good after complete removal of the pancreas. Others have found that completely removing the pancreas may have a much more marked effect on fat absorption than diverting its secretion or leaving a fragment, possibly because it has an internal as well as an external secretion, and possibly because when any of the pancreas at all is left in place there is no certainty but that some of its secretion may reach the intestine (Holmberg, 1911). The failure to absorb fat in normal amounts appears not to be due to incomplete hydrolysis, for that is generally complete (Minkowski, 1890), but rather to the fact that as a result of a too-slow hydrolysis, the absorbing vehicles, the bile salts, are absorbed before complete hydrolysis of the fat has taken place.

The work of Jansen (1911) on the effect of internal secretion of the pancreas on fat absorption has been mentioned. As long as there was any pancreas left, even if it had been transplanted, fat absorption con-

tinued good, but removal of the last fragment reduced absorption to 30-57 per cent. Lombroso (1908) obtained similar results by leaving the uncinate process in place but allowing the excretion to escape by a fistula. Terroine (1919) raised the objection originally advanced by Hédon (1897) to this type of experiment, that the organism had time (about a month) between the operations for considerable adaptation, the intestinal lipases taking over the functions of the pancreatic lipase. With regard to the question of the effect of internal secretion of the pancreas on fat absorption as raised by the work of Jansen (1911) and Lombroso (1908), it should be noted that in cases of complete removal there is also a partial failure of the absorption of other foods (Lombroso, 1908) which probably had an influence on fat utilization.

In connection with partial removal experiments, it should be noted that the factor of safety in pancreatic secretion is relatively large. Brugsch (1906) found that when all the pancreas but a fragment 2 to 3 cm. long and 1.5 cm. wide was left in connection with the duct, absorption of milk fat was 80 per cent complete; and in our own experiments (Bloor, Gillette and James, 1927) with depancreatization of dogs for experimental studies in diabetes, it was found that eight-ninths of the pancreas could be removed without noticeable effect either as regards digestion or carbohydrate metabolism.

Taken altogether, there is no doubt that the pancreatic lipase is a very important factor in fat digestion and absorption, but its functions can be taken over to a considerable extent by other agents.

Bile. As discussed earlier, bile is perhaps the most important single factor in fat absorption. It activates the pancreatic lipase, aids in emulsification, and is a solvent and vehicle for the fatty acids during absorption. Rachford (1891) found that bile greatly increased the activity of pancreatic juice on fats, a result which has been confirmed by many others. An interesting fact brought to light by Loevenhart and Souder (1907) is that the amount of bile needed for maximum activation of the pancreatic lipase varied with the different esters used. Thus, for triglycerides of the higher fatty acids, the optimum concentration of bile salts was 2 to 4 per cent, whereas for simple esters the value was 0.1 per cent. However, a satisfactory amount of fat splitting can take place without bile. In a metabolism study of a four-months' infant with congenital atresia of the bile ducts, Hutchinson and Fleming (1920) found that on a fat intake of 19.6 grams per day only 13.1 per cent was absorbed. The feces fat averaged 65.5 per cent of the dry feces weight as compared with a normal value of 33.3 per cent; yet the degree of fat splitting was 86.9 per cent, only slightly lower than the normal value of 95.2 per cent. Hence the absence

of bile interfered with the lipolytic action of the pancreatic secretion to only a small extent.

Absorption of Fats

Form in which Fat Leaves the Intestine

As has been seen, the provisions for the hydrolysis of fat in the digestive tract are so adequate that, considered along with the fact that absorption of the products of hydrolysis is going on at the same time, it is unlikely that any of the fat normally escapes hydrolysis. Even when one of the important factors, such as bile or pancreatic juice or both, is lacking or insufficient, splitting is practically complete, even though absorption may not be. Under these circumstances, there seems to be but one answer to the question as to the form in which fat is absorbed: that is, it is absorbed as the split-products; and the facts render almost academic the discussion of the absorption of unsplit fat. On the other hand, the structure of the absorbing surface of the intestine; the presence there, in large numbers, of leucocytes which are generally loaded with fat and which are known to pass through the intestinal epithelium; the fact that non-motile bacteria pass across the intestine when fed along with the fat, and not otherwise; the histological picture of the epithelial cells during fat absorption, which fits in with the conception of the absorption of unsplit fat in an ultramicroscopic state of division; together with the fact that mainly neutral fat is found in the lacteals and thoracic duct—all these make it unsafe to assume that fat is absorbed wholly in the hydrolyzed form, and render desirable careful consideration of the possibility of the passage of unaltered fat across the intestinal wall. Therefore, a brief review of the evidence for both points of view is given.

Absorption of split products

That the split products of fat were absorbable was demonstrated very early in the study of the behavior of fat in the intestine. Radziejewski (1868, 1872) showed that alkali soaps were absorbed. Perewoznikoff (1876) showed that a mixture of alkali soap and glycerol was absorbed and synthesized into fat, the lacteals having the same appearance as after a fat meal and the epithelial cells containing fat globules. All later evidence confirms the earlier findings (Munk and Rosenstein, 1891; Bang, 1918b), and it may be regarded as proved that the intestine can absorb fatty acids or soaps and glycerol and synthesize fat from them. Evidence presented by Jeker (1936) indicates that fatty acids might be present in the epithelial cells as soon as 10 to 20 minutes after fat feeding, and that at the sixth hour the cells were filled with neutral fat while the fatty acid

had disappeared. Unfortunately, however, Jeker used histological staining methods, which, in view of the critical work of Kaufmann and Lehmann (1926) are of doubtful significance with respect to the fatty acids. Terroine (1919) has shown that the rate of hydrolysis of fats by pancreatic juice *in vitro* is in direct relation to their rate of absorption in the dog, as shown by blood fat studies. In other words, the rate of absorption is determined by the rate of hydrolysis. Kohl (1938), using a labelled fat, trielaidin, found in rats that fat was absorbed at a nearly constant rate until practically all was absorbed.

A number of workers have experimented with fatty substances other than fats which are hydrolyzable and which yield fatty acids in the intestine, in the hope of obtaining information regarding the manner of absorption of fat. Their results support the conception of the absorption of fat as the split products. Ethyl esters of the fatty acids (Frank, 1898), amyl esters of the fatty acids (Munk and Rosenstein, 1891), and optically active mannite esters of the fatty acids (Bloor, 1912) were all found to be hydrolyzed in the intestine, and the fatty acid components appeared in the chyle as triglycerides, showing that a synthesis with glycerol had taken place. The feeding of the incomplete glycerides, *e.g.*, monoglycerides, similarly resulted in the formation of complete triglycerides (Frank, 1898), indicating complete hydrolysis.

Lyman (1917) has compared the absorption of palmitic acid with that of some of its esters in the rat. He found that glycerol palmitate was absorbed to the extent of 95 to 96 per cent; palmitic acid, 80 per cent; ethyl palmitate, 59 per cent. In all cases, the fat deposited in the tissues was tripalmitin. Of the three, ethyl palmitate is the only one which is liquid at body temperature and it was least well absorbed.

That there is a considerable factor of safety in most living mechanisms and processes is too well known to need discussion. It is interesting to know how much of the ordinary intestinal length is necessary for the utilization of the normal amount of fat in the food. Answers to this question are given in part by necessary operations on humans and in part by experimental operations on other animals. Schumm and Papendieck (1923) reported a study of the feces of a patient after recovery from an operation in which about 370 cm. (about 12 feet) of small intestine were removed. The feces amounted to about one kilo per day and contained two-thirds of the ingested nitrogen and four-fifths of the ingested fat. The feces fat contained 24 per cent neutral fat, 63.6 per cent free fatty acids, and 6.9 per cent fatty acids as soaps. It is to be noted in this case that there was a good hydrolysis, as in most others in which absorption of fat was defective.

Absorption of unchanged fat

There is no good evidence that fats can be absorbed unchanged in any considerable amount. The belief that such was the case was based largely on the observation that the appearance of the fat in the intestine and the lacteals was the same—a milky emulsion. The most important investigations on this subject are those of Schäfer (1885) and of Heidenhain (1888, p. 95), who, however, arrived at opposite conclusions as to the way in which the fat passes across the intestinal walls. Work by Clark and Clark (1917), while not directly concerned with fat absorption from the intestine, is still of great importance in its consideration.

Schäfer (1885), in discussing the phenomena occurring in the intestinal epithelium during fat absorption, first noted that there is no definite, or at least no rigid cell membrane surrounding the columnar epithelial cells of the villi, since soft-bodied cells, such as leucocytes, are able to indent the epithelial membrane and to work their way through between the cells. During fat absorption, the epithelial cells become filled with fat globules of various sizes, generally largest in the part between the nucleus and the thickened border, and often quite small near the attached end of the cell. Sometimes the greater part of the fat is accumulated in the inner, sometimes in the outer part of the cell, these conditions probably representing the different stages in its absorption. The appearance shown in different preparations is such as to indicate a primary accumulation of fat in the outer or free half of the cell and its gradual passage down into the inner or attached half, accompanied by the breaking down of the larger particles into smaller ones preliminary to passage out of the cell. From this histological picture, the conclusion would be that the fat is absorbed in some invisible form, perhaps as very finely divided fat but more likely as the split products, and that it is built up again into visible particles in the cell. Finely divided fat was found by Kitagawa (1934) to be absorbed by the large intestine and lower small intestine of dogs without splitting.

Heidenhain (1888, p. 82) differs from Schäfer in some important particulars as to his explanation of fat absorption. He admits that the leucocytes undoubtedly can take up fat from the intestine, but believes their part is secondary for the following reasons. (a) In newborn puppies, when fat absorption is in full progress, leucocytes are seldom found in the epithelium, whereas in fasting they are often present in large numbers. (b) Leucocytes containing granules which stain black with osmic acid are often found in the Lieberkühn's glands, and their presence is difficult to explain since there is no apparent reason why fat should be carried there. (c) Granules staining black with osmic acid may be found in fasting animals near Lieberkühn's glands and in fewer numbers in the gland itself,

although, on the other hand, evidence is brought to show that probably some substance other than fat may be responsible for the color with osmic acid.

Heidenhain is inclined to regard the presence of fat between the epithelial cells observed by some workers as due to muscular contractions of the villi during fixing, since it cannot be observed in the epithelial cells of animals, such as the frog, whose villi have no muscle fibers.

The work of Clark and Clark (1917) (see p. 96) indicates that leucocytes can take up unsplit fat and transport it fairly rapidly; hence, their presence in the intestinal mucosa in such large numbers during fat absorption and the fact that they are always loaded with fat, even when the epithelial cells contain very little, indicate that they may have a considerable part in one stage of the absorption, *i.e.*, transport to the lacteals. But numerous as they are in the lacteals and around the epithelial cells, they rarely get out into the lumen of the intestine, so that the first stages of the absorption of fat must take place in the epithelial cells themselves.

The striated outer border (next to the lumen) has been the object of a good deal of interest in connection with the passage of fat into the epithelial cells. A few investigators claim to have observed what appeared to be ameboid protrusions from the cell borders into the intestine, and have ascribed to these processes the function of engulfing fat particles from the intestine and transferring them to the interior of the cell. This idea of the method of entry of fat particles into the cells is given sufficient credence by histologists to be included in at least one modern textbook. However, the possibility that the epithelial cells act phagocytically, engulfing the unchanged fat particles, seems doubtful from the fact that fat particles have never been observed in the cuticular membrane, and that most of the considerable mass of evidence available points to the absorption of fat in the form of its hydrolytic products: glycerol and fatty acids (or soaps). Nevertheless, the question as to the passage of unsplit fat from the intestine keeps coming up. Wotton and Zwemer (1939) show photographic records of what seem to be fat globules in hour-glass form partly in and partly out of the epithelial cells during fat absorption, the striated border acting as a narrow gateway through which the globule is working its way. On the other hand, there is nothing to indicate whether the globule is unsplit fat or fatty acid.

The fact that the particles of suspended fat in the lacteals are much smaller than those in the intestine, which has been used in support of the direct absorption hypothesis, has never been explained, although it is reasonable to say that resynthesized fat would very probably be present in finely divided form, since it was synthesized in molecular form. Hydrolysis of fat by the lipases in the intestine was never denied by the

supporters of the theory that fat was absorbed as such, but their claim was that only enough hydrolysis took place to produce soap for emulsification. The repeated statements of the histologists that no fat particles could be observed in the cuticular membrane, which is the first line of action of the epithelial cells, has never been answered; it can be explained only by the assumption that the fat particles are so fine as to be ultramicroscopic. There appears to be nothing inherently impossible in the reduction of fat particles to ultramicroscopic size, since fat is known to occur in a colloidal condition in nature such as stored material in yolk of egg and in many cells, both plant and animal; also, as noted above, the reduction in size of fat particles to invisibility has been observed in leucocytes and in intestinal epithelial cells. But the ability to reduce the fat to particles of ultramicroscopic size appears to be confined to the living interior of cells and probably does not take place in the intestine.

Even if it were possible to reduce fat to the colloidal condition in the intestine, a special mechanism must still be assumed for its absorption, since other substances in colloidal suspension, such as proteins and starches, do not pass into the blood unchanged. Furthermore, it has been shown that non-hydrolyzable substances of a fatty nature which can be emulsified are apparently not absorbed. Two substances have been used for this purpose: esters of the fatty acids with cholesterol or related substances, such as lanolin (yielding a fine emulsion with water), and the paraffin hydrocarbons, which are soluble in fat and can be emulsified with it. Absorption of these substances has been tested in two ways: by determinations of unabsorbed residue in the feces (Henriques and Hansen, 1900; Connstein, 1899), and by determining the absorbed substance in the chyle (Bloor, 1913). In neither case was there evidence of absorption. A similar rejection of paraffin hydrocarbons was observed by Clark and Clark (1917) in their study of the absorption of fat and fat-like substances injected into the tails of tadpoles. Later work by Channon and Collinson (1929) indicated a small absorption of the paraffins. Channon and Devine (1934) found that *n*-hexadecane is absorbed by cats to the extent of about one gram per day, and can be demonstrated in the unsaponifiable matter of tissues, such as the perirenal fat, muscle, and skin, but not in the liver. They concluded that the cat can metabolize *n*-hexadecane. The presence of squalene ($C_{30}H_{50}$), an unsaturated hydrocarbon, in large amounts in shark liver is apparently another example of the ability of an animal to metabolize hydrocarbons. Twort and Twort (1933) found fatty infiltration of the liver of mice following the ingestion of mineral oils.

Other evidence of the absorption of unchanged fat, such as the absorption of colors which are soluble in fat but not in water, and the deposition

in fat stores under certain conditions of large amounts of fat which is chemically the same as the fat of the food, can be explained by the hydrolysis-synthesis theory, since the fat dyes used are soluble in fatty acids (Moore, 1903; Mendel and Daniels, 1912), and since the fat found in the lacteals and hence in the blood and fat stores must be built up largely from the available hydrolysis products of the fat in the intestine. The passage of tubercle bacilli through the intestinal walls, which according to Ravenel (1903) is aided by fats, may also be due to their solubility in fatty acids.

Work on isolated portions of the intestine should be of interest in this connection. Early experimenters in this field were Fürth and Schütz (1907), who obtained the following results in intestinal loops: Stearic acid soaps were very poorly absorbed, only 15-20 per cent; oleic soaps, 15-50 per cent; oleic acid, over 40 per cent; and olive oil, much better than oleic soaps. Absorption of soaps was not helped by glycerol; indeed the absorption of stearic acid soaps was hindered, and no regular improvement of absorption of free oleic acid or olive oil by it was noted. Pancreatic juice and bile together did not improve absorption. In general, they considered absorption to be relatively poor and regarded testing of absorption by this method as unsatisfactory. Later, von Fekete (1911), making use of a Thiry-Vella loop, found that lanolin, lecithin emulsions, and olive oil-lecithin emulsions were not absorbed to any extent. Yamakawa, Nomura, and Fujinaga (1929) reported that if a fine oil-lecithin suspension is injected into the tied-off large intestine of dogs, there is complete absorption in 24 hours. However, Verzár (1933) repeated these experiments with negative results. He was also unable to repeat the results of Mellanby (1927) on the absorption of unsplit fat, but he was able to show that the presence of bile acids was necessary, not only for splitting but for absorption. On the other hand, Doubilet and Reiner (1937) tested a loop of ileum for fat-absorbing ability and found that olive oil and oleic acid were absorbed without the aid of bile salts; a considerable volume of fluid was secreted by the loop, and this was increased by the presence of bile salts. In a jejunal loop in dogs, Riegel, Elsom and Ravdin (1935) found that the absorption of oleic acid was negligible without bile salts. The conflicting nature of these data may perhaps be accounted for by two factors: (1) The action of intestinal lipase was not eliminated in all these cases, even though pancreatic lipase was ruled out. (2) The possible action of bacteria in some cases was not taken into account. Tileston (1912) mixed butter with normal moist feces and obtained a splitting of 12-14 per cent in 24 hours, which, in view of the fact that the fat was not emulsified, is probably to be regarded as a rather rapid hydrolysis.

Summary

Abundant facilities are provided for the hydrolysis of the fats and of those esters of the fatty acids which hydrolyze with the same or greater ease than the fats. The split products are readily absorbed and converted into fat in the passage through the intestinal wall. Substances which cannot be hydrolyzed and so rendered water-soluble, with the exception of cholesterol and possibly other sterols for which another mechanism is available, and probably hydrocarbons in small amounts, are not absorbed, no matter in what form they may be presented. The prevailing belief that fats are completely hydrolyzed in the intestine and absorbed as the hydrolysis products thus has most of the evidence in its favor, although the possibility of the absorption of some unchanged fat cannot be absolutely excluded. The reason for the hydrolysis, which appears to be universal for all food substances, is not far to seek. On the one hand, there is the necessity for the exclusion of substances which, as presented, would be useless or harmful to the organism, such as unchanged proteins, complex carbohydrates, or fat-like substances other than fat. On the other hand, since few food substances are ingested in immediately usable form, more or less hydrolysis must take place, if not in the intestine, then in the tissues. Two purposes are combined in the intestine, which by the same process rejects harmful or useless substances and reduces the useful material to fragments which can be used at once by the tissue cells which require them. There is therefore no need to assume an unusual mechanism for the transfer of fat from the intestinal lumen into the epithelial cells; this takes place according to the usual rule, by hydrolysis as far as needful, followed by absorption of the split products in water-soluble form. The resynthesis of the split products into neutral fat and probably phospholipid, as carried out in the intestinal cells, is probably necessary because of the hemolytic activity of the fatty acids. The resynthesis also allows a rearrangement of the fatty acids in the triglyceride molecule.

The bile salts, according to the hypothesis of Verzár and Kúthy, are hydrotrophic substances which are adsorbed on the surface of the intestinal mucous membrane and aid the passage of fatty acids and cholesterol. Other substances, such as phospholipid, may have the same accelerating effect on fat passing in and out of cells. The phosphorylation of the fat in the intestinal cells appears to be a stage in the resynthesis of fat.

Resynthesis of Fat

A corollary to the fact that fats are hydrolyzed in the intestine and found in the chyle in the resynthesized glyceride form is the necessity that they be resynthesized during their passage through the epithelial cells

(von Walther, 1890). Direct information as to the mechanism of such a resynthesis is accumulating. Several workers have experimented with the synthetic powers of hashed mucous membrane without success. Moore (1903) reported that during fat absorption, the fatty acids in the mucous membrane amounted to 15-35 per cent of the total fat, whereas in the mesenterial glands and lymphatics it amounted to only about 4 per cent, a finding which he believed to indicate that synthesis of fat was taking place in the mucous membrane. Sinclair (1929) was unable to corroborate this difference; but while working on this part of the problem he happened on what seems to be the real mechanism of the resynthesis. He found that, although the phospholipid content of the mucosa did not change during fat absorption, the fatty acids of the phospholipid of the intestinal mucosa changed in response to the fatty acids which were being absorbed. His conception of what was taking place is expressed in the diagram:

Fatty acids ⟶ Phospholipid ⟶ Neutral fat

As soon as the fatty acids are absorbed into the epithelial cells, molecules of phospholipid react with the free fatty acids (or soaps) to form neutral fat; immediately, however, the residual phosphoric acid-base complex unites with the newly absorbed fatty acids and glycerol to form phospholipid, thereby maintaining the amount constant although the nature is changed. The absorbed fatty acids and glycerol thus pass through the phospholipid stage in the intestinal mucosa on their way to neutral fat. The introduction of fatty acids from the food into the phospholipid of the mucosa was first shown by the use of cod liver oil. The replacement of the cod liver oil fatty acids in the phospholipids was shown by feeding beef tallow (Sinclair and Smith, 1937), the fatty acids of which replaced the cod liver oil fatty acids about as rapidly as these had appeared in the mucosal phospholipid. Other evidence of phosphorylation of the neutral fat during absorption has been noted (see p. 93).

Typical experiments from Sinclair's work were as follows: Two cats of about the same weight were kept together on the same diet for at least four days. One was killed 18 to 24 hours after the last meal while the other was fed 25 to 30 grams of cod liver oil and killed 6 hours afterward. The mucosa and muscle of the small intestine of both animals were separated, hashed, and extracted with hot alcohol in a continuous extractor. The phospholipids were separated and purified by acetone precipitation, according to the procedure of Bloor (1926). The total acetone-insoluble material, part of which was soluble in ether and part insoluble, was saponified for 3 to 4 hours. After acidification, the fatty acids were extracted with petroleum ether. Either the whole amount or suitable

aliquots were dried on a steam bath with a current of carbon dioxide, weighed, and the iodine absorption number determined.

The average amount and the iodine number of the phospholipid fatty acids contained in the mucosa and muscle of the small intestines of five pairs of cats are given in Table 5.

Table 5. Average Amount and Iodine Number of the Phospholipid Fatty Acids from Small Intestine (Sinclair, 1929).

	Phospholipid Fatty Acid		
	Moist Tissue (%)	Dry Tissue (%)	Iodine Number
Mucosa			
Control cats, postabsorptive	1.13	7.44	94 ± 2.1
Cats fed cod liver oil about six hours before death	1.15	7.21	107 ± 1.5
Muscle			
Control cats, postabsorptive	0.59	3.06	97 ± 1.4
Cats fed cod liver oil about six hours before death	0.64	3.37	99 ± 0.6

Since there is no change in the amount of phospholipid present, the change in iodine number of the phospholipid fatty acids must signify that part of the fatty acids of the phospholipids within both the mucosa and muscle of the small intestine have been replaced by those of the ingested cod liver oil. In the mucosa this replacement is very uniform and amounts to 16 per cent on the average; the change in the muscle phospholipids is much less uniform and amounts to only 7 per cent on the average.

This change in the nature of the epithelial phospholipids under the influence of the absorbed fat is a probable step in the mechanism of the resynthesis of fat in the epithelial cells of the mucous membrane, and also offers an explanation of the change in the nature (iodine number) of the fatty substances which has been observed during fat absorption. It also offers an explanation of the fact noted by Smith and Rettie (1928), as the result of histological studies, that the fatty material passes through a stage of invisibility immediately after entry into the epithelial cells. In this connection, it is interesting to note that Cramer and Ludford (1925) have claimed that the Golgi apparatus of the epithelial cells (believed to be lipid in nature) is concerned in fat synthesis.

Sinclair's conclusion that phosphorylation is a step in the absorption of fat has been abundantly supported by later work. Süllmann and Wilbrandt (1934), from Verzár's laboratory, found a considerable increase of phospholipid in the intestinal lymph during fat absorption. Verzár and Laszt (1934a) found that, when oleic acid and bile salts were introduced into a loop of rat intestine, absorption took place at a fairly rapid rate which was not affected by glycerol or phosphate alone, but that, when the two were supplied together, and especially when supplied as glycero-

phosphate, absorption was greatly increased. Monoiodoacetate abolished the absorption. In later experiments (1934b), the absorption of olive oil by mouth was totally abolished by iodoacetate and by phlorizin. Since these substances are known to inhibit the esterification of hexoses with phosphoric acid, it was assumed that they also inhibited the phosphorylation of the fatty acids. Laszt and Verzár (1936a) found that extirpation of the adrenals kept the fatty livers from being poisoned by phosphorus and also prevented the removal of fat from fatty livers, from which they concluded that fat mobilization and phosphorylation were controlled by the adrenals. This conclusion is supported by later work (Laszt and Verzár (1936b) in which it was found that adrenal cortical extract reestablishes fat absorption in adrenalectomized animals as does also flavine phosphate. With these and with yeast, fat transport to the liver was reestablished in adrenalectomized rats poisoned with phosphorus. Supplying salt with the food was found by Barnes and associates (1939) to result in normal fat absorption in adrenalectomized animals.

Himwich and his associates (1934) find a moderate rise in the lipid phosphorus of the thoracic duct lymph during fat absorption.

Various experimenters with the radioactive isotope of phosphoric acid have shown that it is promptly combined into phospholipid in the intestinal mucosa, and the combination is faster if fat is given with it. Even in blood *in vitro*, the formation of phospholipid has been shown to take place by Hahn and Hevesy (1938), who found that by shaking blood for 4.5 hours with radioactive phosphate, some radioactive phospholipid could be demonstrated. The amount was small (about 0.3 mg. per cent) but definite. The formation took place faster in the corpuscles, but was definitely demonstrable in plasma freed from corpuscles. Formation of phospholipids in the blood can account only to a small degree for alimentary lecithinemia.

Other evidence regarding the phosphorylation of fat during absorption has appeared from several quarters. Artom and Peretti (1935) found in the intestinal mucosa of rats after large doses of iodized fat that part of the iodized fatty acid was in the phospholipid fraction. The amount fell gradually after the first hour. Sinclair, using a "tagged" fatty acid (elaidic acid in elaidin), was able to follow the absorbed fatty acids and to confirm his earlier results on phosphorylation as a mechanism in fat absorption. Perlman, Ruben and Chaikoff (1937) fed radioactive phosphorus (P^{32}) to rats with and without cod liver oil, and found the active phosphorus in the intestinal phospholipid to be maximum at 12 hours, and that there is a much higher maximum with cod liver oil than without it. The active phospholipid maximum occurred rather later in the liver than in the intestine and was independent of the oil fed, indicating a source

of fatty acid other than that from the intestine. Hevesy (1938) made a number of experiments with P^{32} and obtained evidence on several points. For example, the formation of phospholipid from the administration of phosphate was greatest in the liver; milk fat did not originate in blood phospholipid; and the increased phospholipid in the blood in lipemia did not originate in the intestine. Artom and associates (1938) found that phospholipid is formed most rapidly in the intestine and liver and least rapidly in the muscles and brain. These results all indicate a ready formation of phospholipid in the intestinal mucosa from the fatty acids of the food fat. Most of the fatty acid incorporated in the phospholipid is changed into fat again, but some undoubtedly reaches the blood as phospholipid, possibly via the portal vein. The factors controlling the form of entry of the fatty acids into the blood and their distribution, whether as phospholipid or as fat, are not known; but it is a short step to the assumption that those fatty acids which are to be used immediately are distributed as phospholipid, and that those which are to be stored are distributed as fat. The liver also forms phospholipid, and presumably this formation, as has often been assumed, is a stage of preparation of the fat for immediate consumption.

Fat Transport

Transfer to the lacteals

Fat may be seen in the intestinal cells for some time; at least this is the picture when much fat is being absorbed. When little absorption is taking place, no such accumulation can be observed. The leucocytes, according to Schäfer's observations, are always full of fat, whether much or little is being absorbed, and his belief was that the function of the epithelial cells was to receive the hydrolysis products, synthesize them, and store the fat until the leucocytes could transport it to the lacteals. Both Schäfer (1885) and Heidenhain (1888) agree that the fat from the epithelial cells is transferred to the lacteals without alteration other than changes in size of particles, although their ideas of the mechanism differ; and, strangely enough, both theories have been supported by additional evidence. A third mechanism which has been proposed is the hydrolysis-resynthesis theory of Loevenhart (1907). As has just been pointed out, phosphorylation introduces another possibility as regards transport, since some of the fatty acid may be transferred as phospholipid.

Musculature of the villi. For the transfer of fat from the epithelial cells to the interior of the villi, Heidenhain (1888, p. 82) believed that contraction of the protoplasm was responsible, as is the case for the transfer of water. Inside the villi, the fat is in relatively coarse globules and does not assume the dustlike fineness of its final form until it reaches

the chyle vessels. A contribution by Verzár (1931) goes to show that the muscularity of the villi is of considerable importance in intestinal absorption, the lengthening and shortening of the muscle fibers serving to pump the absorbed material out of the villi and possibly aiding in its intake by them.

Transfer by leucocytes. Schäfer (1885) proposed the theory that fats are transferred by the leucocytes from the mucosal cells to the lacteals. Clark and Clark (1917) have presented some experiments which are of great interest in connection with this possible role of the leucocytes in fat transport in the intestine and elsewhere, and furnish support for the claims of Schäfer regarding this function. Drops of fat or fatty acid 30 to 70 μ in diameter (olive oil, cream, yolk of egg, oleic acid) were injected into the tissue of the tails of tadpoles and the resulting reactions noted. In the case of olive oil, soon after injection, leucocytes were observed to pass through the walls of the nearby blood vessels and to wander toward the oil droplet. On reaching it, they flattened out, formed a ring about it and in a few minutes became pigmented, the pigment being apparently minute particles of oil. Lymph vessels grew out to the oil in from a few hours to two or three days depending on the distance, and remained in contact with the oil and the leucocytes for several days. No pigmented leucocytes were seen to enter the lymphatic vessel, but the oil droplets in the leucocytes in contact with the lymph vessel became gradually smaller until the cell became clear. Fine, free fat droplets were engulfed by leucocytes, were then reduced in size and replaced by minute pigmented droplets.

In the case of oleic acid, within a minute or two after injection, the clear globule became opaque and granular and of a brownish color by transmitted light. The leucocytes responded more quickly and in larger numbers than with olive oil (irritation by the free acids?) forming a ring several layers deep, and soon becoming deeply pigmented. The lymph vessels responded as with the olive oil, and a study of the leucocytes showed that they were continually moving away from the fatty acid, wandering up to a nearby lymphatic and in 15 to 30 minutes moving away, having lost their brown pigment. None was observed to enter the lymph vessel. Absorption of oleic acid or sodium oleate was more rapid than olive oil.

When cream or yolk of egg (containing much finely divided fat) was injected, the same reactions were observed, the leucocytes acting as carriers but working in this case more rapidly. When injection was near a lymph capillary, loaded leucocytes came into contact with the lymphatic within three hours after the injection. If, however, the leucocytes had far to go they lost their pigment before reaching the lymph vessel; which,

however, continued to grow in their direction and that of the fat mass, indicating perhaps a diffusion of soluble and therefore invisible products (hydrolysis products?) from the leucocyte. The absorption of cream or yolk of egg was so rapid that there was often no time for the lymphatics to grow out to the injected material. Leucocytes and lymphatics were the only structures reacting to the injected fat, the blood capillaries, if anything, growing away from it. These reactions were limited to fat and fatty acid. No reaction could be obtained with fat-like substances such as mineral oil.

Considering this work and the fact that leucocytes are present in the intestinal mucosa in such large numbers during fat absorption and are always loaded with fat even when the epithelial cells contain very little, it is reasonable to suppose that the leucocytes may have a considerable part in the transport of fat to the lacteals.

Hydrolysis-resynthesis theory. Loevenhart (1907), on the basis of chemical evidence, believed that the passage out of the intestinal cells, like the passage into them, was accomplished by hydrolysis and synthesis, and that wherever in its subsequent history fat had to pass a cell wall the same hydrolysis and synthesis took place. His assumption required the presence at all these points of a sufficient supply of lipase to bring about these changes in the time ordinarily consumed in transferring the fat from the intestine to the tissues; and he brings evidence to show that lipase is present in all tissues, especially in those which are ordinarily most concerned in fat metabolism: the liver, active mammary gland, blood, lymph, and intestinal mucosa. He noted particularly its presence in those places where fat synthesis is known to take place: the active mammary gland and the subcutaneous fatty tissue. However, the test employed by him to show the presence of lipase was the ability of a water extract of the tissue to hydrolyze ethyl butyrate. Aside from the fact that the splitting obtained by him was rarely very extensive, being ordinarily less than 5 per cent in 40 hours and therefore probably unimportant as a factor in fat metabolism, his experiments have aroused critical comment in several quarters. Regarding the use of ethyl butyrate as a test of the presence of lipase, it was pointed out by Arthus (1902) and later by Jansen (1911) and Foá (1915) that a test carried out on the esters of the lower fatty acids, such as monobutyrin (and therefore also ethyl butyrate), cannot give a true measure of lipase action. Bradley (1913a,b) was unable to corroborate Loevenhart as to the presence of true lipases at the necessary points, such as the mammary glands and the fat-storing tissues.

The lymph in fat transport

The part played by the lymph in collecting and transporting fat from

the absorbing surfaces in the intestine to the blood stream is well known and needs no comment. That it is also of importance in the transport of fat during fasting is rendered probable by the work of Rony, Mortimer and Ivy (1932). They found that the lymph of fasting or phlorizinized dogs contained more fat, but less sugar, than the blood plasma at the same time. After 2 to 14 days' fast, the total fatty acids varied from 250 to 1030 mg. per 100 cc. of lymph, and from 157 to 371 mg. per 100 cc. of blood. The lymph fat is principally derived from fat reserves and not from the blood. That the intestine is the immediate source of the chyle fat in these fasting animals was indicated by the fact that after removal of the intestinal tract the values in the lymph were low (1933). They believed that there is a secretion of fat into the intestine in fasting, the purpose of which appeared to be partly to transport excreted sterols, but perhaps mainly to allow a making-over of the depot fat by the intestinal mucosa. The fact that bile was found necessary for the reabsorption is in line with Sperry's (1927) observation that in bile fistula animals the lipid excretion is much higher, and with that of Beumer and Hepner (1929), who found that there was a larger excretion of cholesterol in a bile fistula dog than in a normal dog. Related to this fact is the old observation of Nikolaides (1899) that the Brunner's and pyloric glands of the stomach show increasing fat content with hunger.

Changes in Fat During Absorption

In spite of the fact that large amounts of food fat may be transported directly to the fat depots without much change, evidence is available to show that under normal conditions, when the animal has free choice of food and when the amount of fat ingested is not too large, the fat in the chyle is noticeably different from the food fat in the intestine. Three factors appear to be responsible for these differences: (a) Selection by the absorbing cells from the food fat of certain fractions (generally the lower-melting); (b) other changes in the nature of the additions of body fat; and (c) chemical changes (saturation or desaturation) which may alter the composition.

With regard to the first factor, selection, Munk (1890) found that, in dogs fed with lard, the fat of the feces had a considerably higher melting point than the fat fed. The difference in melting point may indicate a preferential absorption of the lower-melting acids, but might just as well be explained as the result of excretion of high-melting fatty acid into the intestine. With regard to the third factor, change during the passage from the intestine, Munk and Rosenstein (1891), after feeding cetyl palmitate, found that the chyle fat consisted of one part of triolein and six parts of tripalmitin, with a melting point of 36°C. Frank (1898),

after feeding ethyl palmitate, found 36 per cent of olein in the chyle fat, and, after feeding mutton tallow (1894) (melting point 51.7°C.), obtained a chyle fat melting at 38°C. Munk and Rosenstein, after feeding mutton fat to their patient with a chyle fistula, found that the chyle fat was several degrees lower in melting point than the fat fed; but since only about 55 per cent of the fat fed was absorbed in seven hours, the difference was probably a matter of choice in absorption in favor of the lower-melting constituents of the mixture. Bloor (1913, 1914a) obtained evidence of an alteration in the other direction, *i.e.*, the chyle fat having a higher melting point than the fat fed. After feeding olive oil, of which the constituent fatty acids had a melting point of 16°C. and an iodine number of 86, chyle fat was obtained with a melting point of 30°C. and iodine numbers as low as 72. Other evidence corroborating the above findings was furnished by Raper (1913), who found that after feeding coconut oil the fatty acids of the chyle fat had a higher melting point than those of the fat fed, indicating probably a selective absorption—the lower-melting fatty acids in this case passing directly into the blood.

In most of these cases, the influence of lipids present in the fasting chyle was excluded, so that we may conclude that the fat may be considerably modified during the process of absorption. (The fat content of fasting chyle is given by Walther (1890) as 0.29 per cent total lipid, of which 0.05 per cent is lecithin. On fat-free food (egg white and starch) the fat content of the chyle was 0.25 per cent, of which 0.09 per cent was lecithin. Himwich and associates (1934) give the following values for normal fasting lymph from the thoracic duct: total fatty acids 450, total cholesterol 190, free cholesterol (about 24 per cent of total) 45, and lipid phosphorus 12 (= 300 phospholipid) in mg. per 100 cc. Reiser (1937) gives the following values for thoracic duct lymph: phospholipid 72 mg. per cent; total cholesterol 28 mg. per cent of which about 20 per cent is free, and neutral fat 312 mg. per cent. The modifications during absorption appear to be purposive, in that in all cases the tendency appears to be toward the formation in the chyle of a fat approximating the properties of the body fat of the animal. As to the significance of those changes, Frank was of the opinion that there was an addition of body fat to the chyle fat, either by way of secretion into the intestine or after the fat leaves the intestine; and this surmise was shown to be a fact by the work of Rony, Mortimer and Ivy (1932), who demonstrated that the thoracic lymph in fasting and phlorizinized dogs contained more fat and less cholesterol than the corresponding blood. That the origin of this fat was from the intestine, and that therefore fat is mobilized to the intestine, was shown by the fact that after removal of the intestine there was no extra fat in the lymph. No extra fat was found in the cervical lymph even during rapid fat

absorption, showing that the blood fat does not enter the lymph system directly. If, as Leathes and Meyer-Wedell (1909) believed, the liver has the power of desaturating the fatty acids, this power may be characteristic of all living cells to some degree, and there is a possibility that the intestinal cells can desaturate or saturate the fatty acids during their passage. This power of desaturation has been claimed for the intestinal cells by Tangl and Behrend (1930). The experiments of Long and Fenger (1917), working *in vitro* with bile and pancreatic juice in which they obtained marked increases in unsaturation of the fatty acids of triolein and tristearin, point in the same direction. A dehydrogenase which desaturated fatty acids was described by Berend (1933). It was prepared by glycerol extraction of pancreas. The mechanism would allow adaptive changes in the fats during absorption. However, the ability of the living animal body to desaturate fatty acids beyond the introduction of one double bond is rendered doubtful by the fact of the fat deficiency disease of Burr, which seems to show that the introduction of a second double bond into oleic acid is impossible, at least for some animals.

Differences between food fat and absorbed fat in the blood were noted by Bodansky (1931). After feeding olive oil, the increase of saturated acid in the blood was greater than that of unsaturated acid. McClure and Huntsinger (1928) found that the increase of fatty acid in the blood after feeding oleic acid was not entirely due to oleic acid. They suggested either desaturation or mobilization of saturated fat.

The lipid of thoracic duct lymph in dogs, after feeding 17-22 grams per kilo of horse fat, gave the following values: total fatty acids, 3.26 per cent, of which soaps were 1.94 per cent, free fatty acids 3.6 per cent, sterol esters 0.37 per cent, phospholipid 2.55 per cent, and oxyacids 8.8 per cent (Artom and Peretti, 1936). The iodine number of the fat had changed from 49 in the fat fed to 74 in the fat of the thoracic duct; mean molecular weight from 283 as fed to 268 in the lymph. There was much phosphorus in some other form than as phospholipid. Erben (1900) found a similar desaturation (from 49 to 74) in the passage of fat through the intestinal mucosa, and found that the mean molecular weight of the chyle fatty acids was about that of palmitic acid. About one-fifth of the chyle fatty acid was found to be hydroxystearic. Phospholipid phosphorus was only a small part of the chloroform-soluble phosphorus, much being acetone-soluble and therefore not phospholipid.

Passage of Fat Directly into the Circulation

Fat absorption is generally discussed only in connection with the passage of fat into the intestinal lacteals and the thoracic duct and so to the circulation indirectly, since this is the main and, up to the present, the

only demonstrable path of absorption. What are the indications that fat may pass into the circulation in other and possibly more direct ways? It has never been possible to recover all the absorbed fat. from the chyle of the thoracic duct (Munk and Rosenstein, 1891; Walther, 1890; Frank, 1892), which would indicate either that some of the absorbed fat had been stored somewhere along the lymph tract or that some had reached the blood by other paths than the thoracic duct. Evidence regarding absorption directly into the blood stream from the intestine is contradictory. Börnstein, under Heidenhain's (1888, p. 95) direction, compared the fat content of the carotid and portal blood at the height of fat absorption and found the fat content of the carotid artery to be slightly higher both in the blood as such, and in the dry residue, than in the portal vein. D'Errico (1906-7) found during normal fat absorption that the total solids and the fat content of the portal vein were always higher than those of the jugular. After ligation of the thoracic duct the fat content of the dry residue of the portal vein was still higher than that of the jugular, but not nearly as high as would be expected. D'Errico's experiments may be criticized from the fact that samples were taken not simultaneously, but from one-half to one hour apart, and that absorption from the intestine was interfered with by the operation since the total solids in the portal vein diminished considerably. His experiments have been repeated by Zucker (1920) with negative results. He ascribed d'Errico's positive results to faulty technic and concludes that no marked participation of the blood vessels in fat absorption can be assumed.

It should be noted, however, that none of the methods used for the determination of fat can be relied on to give results accurate to within more than 95 per cent of the true value, and in view of the extensive circulation through the portal system a difference of two or three per cent in the fat content would account for a considerable absorption. Eckstein (1925) in many of his experiments on fat feeding with diversion of the thoracic lymph did find slight increases in blood fat and concluded: ". . . experiments adequately favor the supposition that there are channels other than the lymph stream which may serve as a vehicle for the transportation of fat into the body." However, he "hesitates to conclude that fat is directly absorbed by the portal capillaries inasmuch as all of the lymphatics may not necessarily be ruled out by merely diverting the thoracic trunk lymph from the body." Roger and Binet (1922), Sicard, Fabre and Forestier (1923), and Cantoni (1928) also report positive absorption into the portal vein, as does Nedswedski (1926), by examination of the blood going to and leaving the intestine, making use of London's technic for tapping the blood vessels. Cantoni found that after a meal of fat, the total fatty acid content of the portal vein was 178 mg. per

cent higher than that of the carotid artery, and cholesterol 22 mg. per cent higher. Nedswedski found that the liver extracted both fat and cholesterol from the portal vein and liberated phospholipid into the hepatic vein. The experiments of Joannovics and Pick (1910) and those of Sinclair (1929) show quite definitely that there is a portal absorption for cod liver oil, while those of Sinclair with olive oil were negative. Sinclair has since (1936) shown that the fatty acids of the blood phospholipids change promptly with the food fat; and, since there is very little phospholipid in the fat in the thoracic duct, the inference is that the phospholipid has entered by another channel, possibly by direct absorption into the blood from the intestine. Raper (1913), in his study of the absorption of coconut oil, concluded that the lower fatty acids were probably absorbed directly into the portal blood.

It seems possible then that there is at times considerable absorption of fat directly into the blood going to the liver, although satisfactory proof is still lacking. An even approximate balance sheet of the absorption of fat is still to be made. It is perhaps significant in this connection that Clark and Clark (1917) found no attraction between blood capillaries and fat or leucocytes such as is exhibited by lymph capillaries. Recent work has shown a definite large formation of phospholipid in the intestinal mucosa; and since relatively little of it has been found in the lymph of the thoracic duct, and since there is a definite rise in blood phospholipid during fat absorption, it is reasonable to suppose that some of the absorbed fat passes into the blood as phospholipid. If there is no direct absorption into the blood, the 30 or 40 per cent of absorbed fat which cannot be collected from the thoracic duct has to be accounted for either by storage in some place along the lymph path or by entry into the lymph or blood systems by other channels than the thoracic duct. The possibility of such connection has been demonstrated. Thus Lee (1922), after ligation of the thoracic duct, found that connections were established between the lymphatic duct and the azygos vein or its branches, and also with the right thoracic duct. He thinks that these unions may be of considerable importance although, when the thoracic duct is tied off, no detectable amount of fat reaches the blood (d'Errico, 1906-7; Bloor, 1914b; Eckstein, 1925). As to the possibility of fat storage along the path of transport, it is present in the epithelial cells and the intestinal leucocytes for some time after it has ceased to be absorbed from the intestine, and it has been found in quite large amounts in the leucocytes in the lymphoid tissue of the intestine (Heidenhain, 1888, p. 82), even during fasting. Nevertheless, the amount that could be stored in this way is probably not great. The direct evidence against absorption into the portal system by

Zucker (1920) may mean only that the methods of measurement were not sensitive enough. The finding of Munk (1902) that during fat absorption there is an accumulation of fat in the liver after a heavy fat meal he interprets as evidence of direct absorption from the intestine into the blood stream; but it could be just as well explained as a deposition from the blood stream secondary to absorption through the thoracic duct in the regular way.

Parenteral Administration of Fat

Subcutaneous or intraperitoneally administered fat (Ziegler, 1921; Mills, 1911) reaches the thoracic duct and the blood. The subendothelial "net" is the place of most marked absorption. Fat transport by wandering cells is relatively unimportant, whereas capillary endothelium is important. Mills reported that emulsified fats could be given hypodermically over long periods of time without injury and were well absorbed by way of the lymphatics. Unemulsified oils were not well absorbed except on a high-protein diet.

Rabbeno (1914) injected homogenized fat and egg yolk intravenously and found that part of the injected material was retained in the blood for several hours. Iodipin (iodized fat) emulsion appears to leave the blood fairly rapidly, only 5 per cent of the iodine being present in the blood after 72 hours; 65 per cent of the iodine injected is excreted in the urine. Nomura (1929) found that intravenous fat disappeared quickly except for a small amount which persisted up to 24 hours. Deposition was most marked in the liver, skeletal muscles, and subcutaneous tissue. Kimura (1929) found that finely suspended fat, when injected intravenously, passed directly to the tissues without going into the lacteals. Holt and associates (1935) have injected homogenized fat directly into the blood stream of infants and have found that it is apparently well utilized. The emulsion contained from 7 to 7.5 per cent lipids consisting of about one-third of fresh egg lecithin and two-thirds of various fats. Much of this emulsion is removed by the liver. Coarse emulsions are largely retained by the lungs and spleen which seem to act as the first line of defense against large foreign particles in the blood.

Digestion and Absorption of Other Lipids

Phospholipids

As regards digestion and absorption of phospholipids, there is significant work on lecithin only. Like other glycerides, it is apparently hydrolyzed and the fatty acids rebuilt into neutral fat during absorption. The small increases which have been reported in the phospholipid of chyle and

blood during the digestion of lecithin and which have been referred to the absorption of unchanged lecithin have also been found after absorption of neutral fat.

Ehrmann and Kruspe (1913) found that bile was more important than pancreatic secretion in the splitting of lecithin. Slowtzoff (1906) found, after feeding lecithin, that an increase of phospholipid could be noted in the chyle. Eichholtz (1924) reported that after feeding 40 grams of lecithin to a dog there was a slight increase in blood lecithin and fat. Eckstein (1925), having in mind possible lecithin synthesis during fat absorption, found that while fat or palmitic acid feeding did not increase the lecithin of the thoracic duct lymph (which agrees with our own unpublished results) ingestion of oleic acid caused an increase of lecithin in the thoracic lymph. The ingestion of lecithin itself caused an apparent augmentation of the phospholipid content of the lymph (increase in ether-soluble phosphorus), but the phospholipid increments were only slight and did not parallel the fluctuations in the total fatty acid content of the lymph, so that the increase was not regarded as significant. Süllmann and Wilbrandt (1934) found that the phospholipids of the intestinal lymph of rabbits greatly increased (up to one-fifth of the total fatty acids) during fat absorption. Whether this extra phospholipid reached the blood via the thoracic duct is not stated. Rewald (1928), after feeding large amounts of lecithin, found 90 per cent absorption over long periods of time. After several months, accumulations of phospholipids were noted in brain, kidney, liver, blood, and adipose tissue. No effect on the health of the animals was noted. The fact that phospholipid constitutes about 3 per cent of hens' eggs and the increasing use of soybean and similar phospholipid as food constituents make the absorption of unchanged phospholipid an important question which has not been satisfactorily answered.

The small amount of experimental work up to the present indicates that phospholipids are treated in digestion and absorption like the related triglycerides, the fats, with perhaps some difference in behavior due to their greater miscibility with water. The fact that the fats, to a considerable extent at least, pass through a phospholipid stage during absorption adds an additional complication, especially to the later stages of the process.

Sterols

Cholesterol

The sterols, cholesterol in animals and the closely related phytosterols in plants, are apparently very necessary for the life of organisms. Since they are less soluble in water than even the fats, the manner of their absorption becomes of special interest. Regarding the conditions attend-

ing the absorption of cholesterol and its esters, the following summary has been taken in part from a review by Knudson (1921) and brought up to date.

The first work of any significance was reported by Pribram (1906), who found that by feeding pure cholesterol or cholesterol esters, an increase of these substances in the blood of rabbits results. The increase was in part, at least, due to free cholesterol, since the serum from such animals showed an increased inhibitory action on hemolysis by saponin, which is produced by free cholesterol alone. Kusumoto (1908) also showed indirectly that cholesterol must be absorbed; he found that 30 per cent of the amount ingested was not excreted through the intestine. Gardner and his co-workers (1909; 1910), in extensive studies on the origin and destiny of cholesterol in the animal organism, came to the conclusion that cholesterol and cholesterol esters are absorbed into the blood, that the cholesterol esters are hydrolyzed before absorption, and that, after feeding free cholesterol, an increase in both free cholesterol and cholesterol esters in the blood occurs. Lehman (1914) found that feeding cholesterol to rabbits resulted in a few hours in an increase of the cholesterol in the blood. Similar results were reported by Ssokoloff (1924, 1925) after feeding cholesterol in oil or as egg yolk; but he found that long-continued feeding did not produce large increases. In a series of experiments on dogs with fistulas of the thoracic duct, Mueller (1915, 1916b) found that cholesterol was readily absorbed and appeared in the chyle. When cholesterol was fed, either in the free form or in the form of esters, there followed an increase in both of these in the chyle, the proportion between the two remaining approximately the same as in normal blood plasma. These results indicate that, either in the lumen or walls of the intestine, processes of hydrolysis or esterification take place, depending upon the character of the material fed. Chauffard, Laroche and Grigaut (1920) reported an increase of cholesterol in the blood far beyond the amount in the fat fed and suggested a synthesis of cholesterol from the fat.

As the result of his own work on cholesterol and cholesterol ester absorption, Knudson (1917) had shown that during the absorption of a neutral fat (olive oil) there was a marked increase of cholesterol esters in the blood but no increase in total cholesterol. Later (1921) he reported that when cholesterol or cholesterol esters, such as palmitate, oleate, or stearate, were fed, there resulted a marked increase in the total cholesterol in the blood but no change in the amount of cholesterol as esters. The cholesterol esters must as a consequence be hydrolyzed in the intestine before absorption and apparently are not entirely resynthesized before passing into the blood. These results do not agree with the observations

reported by Gardner and his co-workers (1909; 1910), a fact which may be due to the lack of fat in Knudson's diets.

The necessity for a solvent, such as fat, for the absorption of cholesterol was first noted by Hoppe-Seyler. Sano (1924) reported six experiments with dogs to determine the absorption of cholesterol under different conditions, and particularly the ratio of combined cholesterol to total cholesterol. The results showed that the presence of fatty acids or of fats is indispensable for the absorption of cholesterol, and that the rate of absorption and the amount absorbed depend on the quantity of fatty acids which accompany it. Cholesterol in the free state passes slowly as such into the chyle and, in the presence of fatty acids, is esterified more or less completely according to the amount of fatty acids present. When there is little free fatty acid, but fats are present in abundance, considerable cholesterol is absorbed in the free state. Leites (1928) found that olive oil or olive oil and cholesterol caused increases in bile cholesterol in one to 24 hours; meat and lecithin within six hours.

Work by Himwich and associates (1934) led to the conclusion that cholesterol aids in the absorption of fatty acids. These workers found that there was a great increase of total fatty acids, a moderate increase of lipid phosphorus and a marked rise of cholesterol, both free and as ester, in the thoracic lymph after ingestion of fat. The increase of total fatty acids was from 450 to 2275 mg. per cent, of lipid phosphorus from 13 to 17, and of cholesterol from 190 to 629 mg. per cent.

The cholesterol in foods is ordinarily absorbed without difficulty since the food generally contains fat enough for this purpose. Mjassnikow (1926) fed to humans a cholesterol breakfast of eight eggs, or about 2 grams of cholesterol, for one or several days. A single dose produced no effect on the blood cholesterol in normal persons, but caused a rise in individuals with nephrosis and frequently in those with arteriosclerosis. Prolonged dosage increased the blood cholesterol slowly in normal individuals. Bürger and Habs (1927a,b), after feeding 5 grams of cholesterol in 100 cc. of olive oil to human subjects, found that the blood cholesterol was doubled and fatty acid increased by five times at the time of maximum height of the absorption curve (4 hours). Free and bound cholesterol were equally well absorbed and the ratio of free to bound cholesterol in the blood was kept constant.

In connection with the need for fat in the absorption of cholesterol, it is interesting to note that fat also seems necessary for the absorption of galactose. Schantz, Elvehjem and Hart (1938) found that rats fed galactose without fat, either as such or as skim milk, excreted a large proportion of it. The addition of fat abolished the losses. Butyric or caproic acids did not affect the excretion, but higher acids did. The

failure to utilize galactose did not appear at once, but only after a few days. The blood sugar on the fat-free milk was much higher (200 mg. per cent as compared with 140) than on the whole milk. Pigs and calves were found to waste 12 to 16 per cent of the galactose on a fat-free diet. No explanation of this peculiarity is as yet available, but it opens a new field for experimentation and speculation.

The probability that the "choleic acid principle" of Wieland and Sorge (1916) applies to the absorption of cholesterol as well as of the fatty acids is pointed out by Gardner and Gaïnsborough (1930), and demonstrated by Wieland and Sorge (1916). Schoenheimer (1924) found that although desoxycholic acid, fed alone, did not raise the cholesterol content of serum, when added to a cholesterol-fat diet it brought about a marked lipemia and cholesterolemia in rabbits and guinea pigs. Hummel (1929) found desoxycholic acid inferior to glycocholic acid in bringing about retention of cholesterol in mice. Wieland and Sorge (1916) found desoxycholic acid the best solvent for cholesterol. They reported that the desoxycholic-cholesterol combination dialyzed through a collodion membrane and that lecithin aided in the solution of cholesterol by bile salts. Fürth and Scholl (1930) found that the diffusion of cholesterol through plates of agar, gelatin, etc. is aided by bile salts, and Breusch (1937) found that cholesterol in bile salts does not diffuse. Loeffler (1928) found that bile acids fed along with cholesterol greatly increased the storage of cholesterol in the liver. Hummel (1929) obtained similar results, but raised the question of a possible transformation of bile acids into cholesterol.

Herbivorous animals differ markedly from carnivorous animals with regard to the way in which they react to cholesterol. Wacker and Hueck (1919) produced increases in both free and bound cholesterol in the blood of rabbits by feeding free cholesterol, but they could not produce any increase in cats and dogs. (On the other hand, Grigaut and L'Huillier (1912) had succeeded in producing increases in the blood of dogs by feeding cholesterol.) Herbivorous animals absorb fatty substances and cholesterol slowly (Bang, 1918a), but when these substances get into the circulation and the tissues, they are much more difficult to excrete. Carnivorous and omnivorous animals readily show lipemia after fat ingestion, but do not readily form the deposits of anisotropic lipids (cholesterol esters) which herbivorous animals do. Results obtained with one type of animal cannot be compared with those from the other without reservation. Herbivorous animals are not naturally accustomed to much cholesterol in their food and apparently have not as good a mechanism for either absorption or excretion of it as have carnivorous animals.

Other sterols

Fraser and Gardner (1910) investigated the absorption of plant sterols and believed they had demonstrated that these are absorbed and changed into cholesterol during absorption. Schoenheimer, von Behring and Hummel (1930) found, on the contrary, that sitosterol and other plant sterols are not absorbed by animals, even when fed along with bile acids; most recent work is in agreement with these findings, although there is some disagreement. In later work, Gardner and Gainsborough (1930) agreed with Schoenheimer that the change of plant to animal sterols during absorption does not take place. Nikuni (1931), working with mice, found that phytosterol was absorbed as well as cholesterol. It is apparent that the matter has not yet been satisfactorily settled, although the weight of evidence is against the absorption of plant sterols by animals.

Schoenheimer, von Behring and Hummel (1930) found that isocholesterol from lanolin was not absorbed. Allocholesterol was absorbed somewhat less readily than cholesterol. Saturation of the double bond prevents absorption; hence neither dihydrocholesterol nor coprosterol was absorbed (Bürger and Winterseel, 1931). Ergosterol was absorbed when irradiated, but not otherwise. Schoenheimer and Dam (1932) and Menschick and Page (1932) found that laying hens can absorb ergosterol in notable amounts.

As regards other solid alcohols, Mancke (1927) fed cetyl acetate to sheep and goats and could find no increase in unsaponifiable substance in their milk fat. Fed to geese, it was absorbed to the extent of 62 per cent, but no cetyl radical could be found in the fat stores. When cetyl acetate was given to rabbits subcutaneously or intrapleurally, the cetyl radical slowly disappeared. Given intravenously in very fine suspension, it could not be found in the depots or organs. Mancke concludes that the cetyl alcohol is probably oxidized to palmitic acid. Channon and Collinson (1928) report that the rat absorbed alcohols in the following increasing order: phytol, oleyl alcohol, cetyl alcohol, cholesterol; this is also the order of their solubility in bile salt solutions.

Summary

For the absorption of cholesterol, at least two factors appear necessary: first, the presence of bile salts, without which there is no absorption, and this means that the liver must be functioning normally; secondly, the simultaneous absorption of fat or fatty acid, without which there is very little cholesterol absorption and the cholesterol ester percentage in the blood is likely to be low, since the esterification of cholesterol appears to take place during absorption. Other sterols than cholesterol are absorbed

by animals with difficulty or not at all. Other solid alcohols are absorbed in proportion to their solubility in bile salts.

Bibliography

Abderhalden, E., "Biochemisches Handlexikon," Vol. 3, p. 178, Berlin, J. Springer, 1911. p. 76
Amaki, J., *Tôhoku J. Exptl. Med*, 5: 13 (1924). p. 73
Armstrong, H E., *Proc. Roy. Soc. London*, B76: 606 (1905). p 70
Armstrong, H. E. and Gosney, H. W., *Proc. Roy. Soc. London*, B88: 176 (1914). p. 68, 70
Arnschink, L., *Z. Biol.*, 26: 434 (1890). p 59, 62
Arthus, M., *J. physiol. path. gén.*, 4: 455 (1902). p. 74, 97
Artom, C. and associates (Sarzana, G. and Segré, E.), *Arch. intern physiol*, 47: 245 (1938). p. 95
Artom, C. and Peretti, G., *Boll. soc. ital. biol. sper.*, 7: 980 (1932); *C. A.*, 27: 342 (1933). p. 60
Arch. intern. physiol., 42: 61 (1935). p 94
Biochim. terap. sper., 23: 249 (1936); *C. A*, 30: 8333 (1936). p. 100
Artom, C and Réale, L., *Bull soc. chim. biol*, 18: 959 (1936). p. 68
Bach, E., *Z. klin, Med.*, 95: 103 (1922) p. 74
Balls, A. K., Matlack, M B. and Tucker, I. W., *J. Biol. Chem.*, 122: 125 (1937). p 61
Bang, I, *Biochem. Z.*, 91: 110 (1918a). p. 107
ibid., 91: 111 (1918b). p 85
Barnes, R. H. and associates (Wick, A. N, Miller, E. S and MacKay, E. M.), *Proc. Soc Exptl. Biol. Med.*, 40· 651 (1939). p 94
Baumann, E. J., *see* Mendel, L B.
Bayliss, W. M. and Starling, E. H., *J Physiol.*, 30: 61 (1904). p 68
Behrend, N., *see* Tangl, H.
Behring, H. v., *see* Schoenheimer, R.
Belfanti, S., Contardi, A. and Ercoli, A, *Ergeb Enzymforsch*, 5: 213 (1936). p 76
Bénech, E. and Guyot, L, *Compt rend soc. biol*, 55· 719, 721, 994 (1903) p 66
Berend, N., *Biochem. Z.*, 260: 490 (1933). p 100
Bernard, C, "Memoire sur le pancreas et sur la rôle du suc pancreatique dans les phenomenes digestifs, particulierement dans la digestion des matieres grasses neutres," Paris, J. B Bailliere, 1856. p. 67, 79
Beumer, H. and Hepner, F., *Z. ges. exptl. Med.*, 64: 787 (1929). p. 98
Binet, L, *see* Roger, H
Bloor, W. R., *J. Biol. Chem*, 11: 429 (1912). p 86
ibid, 15: 105 (1913). p 89, 99
ibid., 16. 517 (1914a). p. 99
ibid., 19: 1 (1914b). p 102
ibid., 68· 33 (1926). p. 92
Bloor, W. R, Gillette, E. M. and James, M. S., *J. Biol. Chem*, 75: 61 (1927). p 84
Bodansky, M, *Proc Soc. Exptl. Biol Med*, 28: 630 (1931). p 100
Boldyreff, W., *Zentr. Physiol*, 18: 457 (1904). p 66, 70
Z. physiol Chem, 50: 394 (1907). p 70
Arch. ges. Physiol. (Pflügers), 121: 13 (1908). p 79
Ergeb Physiol., 11: 121 (1911). p 66, 79
Boldyreff, W. N. and Kellogg, J. H., *Arch. Internal Med.*, 34: 726 (1924). p 79
Bournot, K., *Biochem. Z.*, 52: 172 (1913); *ibid.*, 65: 140 (1914) p 71
Bradley, H. C, *J. Biol. Chem*, 6: 133 (1909). p. 68
ibid., 13: 407 (1913a). p. 77, 97
ibid, 13: 431 (1913b). p. 97
Brand, E., *see* Sandberg, M.
Breusch, F. L., *Biochem. Z.*, 293: 280 (1937). p. 107
Brockett, S. H., *see* Himwich, H. E.
Brugsch, T., *Z. klin. Med.*, 58: 518 (1906). p. 84
Bürger, M. and Habs, H., *Klin. Wochschr.*, 6: 2125, 2221 (1927a); *Z. ges exptl. Med*, 56 640 (1927b) p. 106
Bürger, M. and Winterseel, W., *Z. physiol. Chem.*, 202: 237 (1931). p. 108
Bylina, A., *Arch. ges. Physiol. (Pflügers)*, 142: 531 (1911). p. 68
Camus, L., *see* Hanriot, —
Cantoni, O., *Boll. soc. ital. biol. sper.*, 3: 1278 (1928); *C. A.*, 23: 5224 (1929). p. 101
Caro, L., *Z. klin Med.*, 89: 49 (1920). p. 73
Carr, R. H., *see* McClure, F. J.
Chaikoff, I. L., *see* Perlman, I.
Chamberlin, H. G., *see* Lockwood, B. C.
Channon, H. J. and Collinson, G. A., *Biochem. J.*, 22: 391 (1928). p. 108
ibid., 23: 676 (1929). p. 89
Channon, H. J. and Devine, J., *Biochem. J.*, 28: 467 (1934). p 89
Chauffard, A., Laroche, G. and Grigaut, A., *Ann. méd.*, 8: 69 (1920). p 105
Cherry, I. S., *see* Crandall, L. A., Jr.
Cherry, I. S. and Crandall, L. A., Jr., *Am. J. Physiol.*, 100: 266 (1932). p. 74
Chrzascz, T. and Janicki, M., *Biochem. Z.*, 296: 295 (1938). p. 68
Clark, E. R. and Clark, E. L., *Am J. Anat.*, 21: 421 (1917). p. 87, 88, 89, 96, 102
Collinson, G. A., *see* Channon, H. J.
Connstein, W., *Arch. (Anat.) Physiol.*, 1899: 30. p. 89
Connstein, W., Hoyer, E. and Wartenberg, H., *Ber. deut. chem. Ges.*, 35: 3988 (1902). p. 70
Contardi, A., *see* Belfanti, S.
Contejean, C., *Arch. physiol. norm. path.* [5] 6: 125 (1894). p. 66
Cramer, W., *see* Mottram, J. C.
Cramer, W. and Ludford, R. J., *J. Physiol*, 60: 342 (1925). p. 93
Crandall, L. A., Jr, *see* Cherry, I. S.
Crandall, L. A., Jr. and Cherry, I. S., *Proc. Soc. Exptl. Biol. Med.*, 28: 570, 572 (1931). p. 74
Arch. Neurol. Psychiat., 27: 367 (1932). p. 74

Dam, H., *see* Schoenheimer, R.
Daniels, A. L., *see* Mendel, L. B.
Dastre, A., *Arch. physiol. norm. path.*, [5] 2: 315 (1890). p. 79
Davis, R. E., *J. Biol. Chem.*, 88: 67 (1930). p. 60
Devine, J., *see* Channon, H. J.
Dissé, W., *see* Kalk, H.
Dobrowitzky, P. J., *see* Wwedensky, N.
Doubilet, H. and Reiner, M., *Arch. Internal Med.*, 59: 857 (1937). p. 90
Drew, A. H., *see* Mottram, J. C.
Eckstein, H. C., *J. Biol. Chem.*, 62: 737 (1925). p. 101, 102, 104
ibid., 81: 613 (1929). p. 60
Eggstein, A. A., *see* Jobling, J. W.
Ehrmann, R. and Kruspe, H., *Berlin. klin. Wochschr.*, 50: 1111 (1913). p. 104
Eichholtz, F., *Biochem. Z.*, 144: 66 (1924). p. 104
Elsom, K. O., *see* Riegel, C.
Elvehjem, C. A., *see* Schantz, E. J.
Erben, F., *Z. physiol. Chem.*, 30: 436 (1900). p. 100
Ercoli, A., *see* Belfanti, S.
Errico, G. d', *Arch. fisiol.*, 4: 513 (1906–7). p. 101, 102
Fabre, R., *see* Sicard, J. A.
Falk, K. G., "The Chemistry of Enzyme Action," 2d ed., pages 137, 178, New York, Chemical Catalog Co., (Reinhold Publishing Corp.), 1924. p. 71, 75
Falk, K. G. and McGuire, G., *J. Biol. Chem.*, 108: 61 (1935). p. 75
Falk, K. G. and Sugiura, K., *J. Am. Chem. Soc.*, 37: 217 (1915). p. 71
Fejer, A. v., *Biochem. Z.*, 53: 168 (1913). p. 82
Fekete, A. v., *Arch. ges. Physiol. (Pflügers)*, 139: 211 (1911). p. 90
Fenger, F., *see* Long, J. H.
Fleckseder, R., *Arch. exptl. Path. Pharmacol.*, 59: 407 (1908). p. 83
Fleming, G. B., *see* Hutchinson, H. S.
Foà, C., *Arch. ital. biol.*, 63: 239 (1915). p. 97
Forestier, G., *see* Sicard, J. A.
Frank, O., *Arch. Physiol. (DuBois-Reymond's)*, 1892: 497. p. 101
ibid., 1894: 297. p. 99
Z. Biol., 36: 568 (1898). p. 86, 98
Frank, P., *Wien. Arch. inn. Med.*, 5: 591 (1923). p. 79
Fraser, M. T. and Gardner, J. A., *Proc. Roy. Soc. London*, B82: 559 (1910). p. 108
Fürth, O. and Scholl, R., *Biochem. Z.*, 222: 430 (1930). p. 107
Fürth, O. and Schütz, J., *Beitr. chem. Physiol. Path.*, 10: 462 (1907). p. 90
Fujinaga, I., *see* Yamakawa, S.
Gardner, J. A. and coworkers, *Proc. Roy. Soc. London*, B81: 109, 230 (1909), B82: 559 (1910) p. 105, 106
Gardner, J. A. and Gainsborough, H., *Quart. J. Med.*, 23: 465 (1930). p. 107, 108
Gillette, E. M., *see* Bloor, W. R.
Glaessner, K., *Z. physiol. Chem.*, 40: 465 (1904). p. 68
Glick, D., *see* Sobotka, H.
Gorbach, G. and Günter, H., *Monatsh. Chem.*, 61: 47 (1932). p. 72
Gosney, H. W., *see* Armstrong, H. E.
Green, J. R., *Proc. Roy. Soc. London*, 48: 370 (1890). p. 70
Greene, C. W. and Skaer, W. F., *Am. J. Physiol.*, 32: 358 (1913). p. 80
Greene, C. W. and Summers, W. S., *Am. J. Physiol.*, 40: 146 (1916). p. 74
Grettie, D. P., *see* Jensen, L. B.
Griffioen, K., *see* Wolvekamp, H. P.
Grigaut, A., *see* Chauffard, A.
Grigaut, A. and L'Huillier, A., *Compt. rend. soc. biol.*, 73: 202 (1912). p. 107
Günter, H., *see* Gorbach, G.
Güntherberg, K., *Wiss. Arch. Landw. Abt. B, Tierernähr. Tierzucht*, 3: 339 (1930); *C. A.*, 25: 5451 (1931). p. 62
Guyot, L., *see* Bénech, E.
Haba, H., *see* Bürger, M.
Hahn, L. and Hevesy, G., *Compt. rend. trav. lab. Carlsberg., Sér. chim.*, 22: 188 (1938); *C. A.*, 32: 6315 (1938). p. 94
Haley, D. E., *see* Longenecker, H. E.
Hanriot, —, *Arch. physiol. norm. path.* [5], 10: 797 (1898). p. 74
Hanriot,—and Camus, L., *Compt. rend. acad. sci.*, 124: 235 (1897). p. 73
Hansen, C., *see* Henriques, V.
Hart, E. B., *see* Schantz, E. J.
Hédon, E., *Arch. physiol. norm. path.* [5], 9: 622 (1897). p. 84
Heidenhain, R., *Arch. ges. Physiol. (Pflügers)*, 43 (Supp.): 1–103 (1888). p. 87, 95, 101, 102
Heinsheimer, F., *Deut. med. Wochschr.*, 32: 1194 (1906). p. 66
Henriques, V. and Hansen, C., *Zentr. Physiol.*, 14: 313 (1900). p. 89
Hepner, F., *see* Beumer, H.
Herter, E., *Z. physiol. Chem.*, 4: 160 (1880). p. 68
Hess, C. L. v., *J. Biol. Chem.*, 10: 381 (1911). p. 73
Hevesy, G., *Enzymologia*, 5: 138 (1938). p. 95
See also Hahn, L.
Hills, H. W., *see* Kernot, J. C.
Himwich, H. E. and associates (Brockett, S. H. and Spiers, M. A.), *Am. J. Physiol.*, 110: 342 (1934). p. 94, 99, 106
Hiruma, K., *Biochem. Z.*, 139: 336 (1923). p. 73
Holmberg, O. J., *Z. physiol. Chem.*, 74: 354 (1911). p. 83
Holmes, A. D., *Boston Med. Surg. J.*, 192: 1210 (1925). p. 60
J. Oil & Fat Ind., 3: 11 (1926); *C. A.*, 20: 2006 (1926). p. 59
See also Langworthy, C. F.
Holt, L. E. and associates (Tidwell, H. C. and Scott, T. F. M.), *J. Pediatrics*, 6: 151 (1935). p. 103
Horiuchi, Y., *J. Biol. Chem.*, 44: 345 (1920). p. 62
Hoyer, E., *see* Connstein, W.

Hueck, W., *see* Wacker, L.
Hughes, R. H. and Wimmer, E. J., *J. Biol. Chem.*, 108: 141 (1935) p. 60
Hull, M. and Keeton, R. W., *J. Biol. Chem.*, 32: 127 (1917) p. 67
Hummel, R., *Z. physiol. Chem.*, 185: 105 (1929). p. 107
See also Schoenheimer, R.
Huntsinger, M. E., *see* McClure, C. W.
Hutchinson, H. S. and Fleming, G. B., *Glasgow Med. J.*, 94: 65 (1920); *C. A.*, 14: 3266 (1920). p. 84
Inouye, T., *Am. J. Physiol.*, 69: 116 (1924). p. 81
Irwin, M. H., *see* Steenbock, H.
Irwin, M. H., Steenbock, H. and Templin, V. M., *J. Nutrition*, 12: 85 (1936). p. 60
Ivy, A. C., *Physiol. Rev.*, 10: 282 (1930). p. 68
See also Lim, R. K. S., Rony, H. R.
Ivy, A. C., Lim, R. K. S. and McCarthy, J. E., *Quart. J. Exptl. Physiol.*, 15: 55 (1925) p. 80
James, M. S., *see* Bloor, W. R.
Janicki, M., *see* Chrzascs, T.
Jansen, B. C. P., *Z. physiol. Chem.*, 72: 158 (1911) p. 70, 83, 84, 97
Jeker, L., *Arch. ges. Physiol. (Pflügers)*, 237: 1 (1936). p. 85
Jensen, L. B. and Grettie, D. P., *Food Research*, 2: 97 (1937). p. 71
Joannovics, G. and Pick, E. P., *Wien. klin. Wochschr.*, 23: 573 (1910). p. 102
Jobling, J. W., Eggstein, A. A. and Petersen, W., *J. Exptl. Med.*, 22: 701 (1915). p. 73
Kalaboukoff, L. and Terroine, E.-F., *Compt. rend. soc. biol.*, 63: 372 (1907). p. 70
Kalk, H. and Dissé, W., *Arch. Verdauungskrankh.*, 33: 117 (1924). p. 79
Kastle, J. H. and Loevenhart, A. S., *Am. Chem. J.*, 24: 491 (1900). p. 66
Kaufmann, C. and Lehmann, E., *Arch. path. Anat. (Virchow's)*, 261: 623 (1926). p. 86
Keeton, R. W., *see* Hull, M.
Kellogg, J. H., *see* Boldyreff, W. N.
Kelsey, F. E., *J. Biol. Chem.*, 130: 187 (1939a). p. 65
ibid., 130: 195 (1939b). p. 65
ibid., 130: 199 (1939c). p. 71
Kemmerer, A. R., *see* Steenbock, H.
Kernot, J. C. and Hills, H. W., *Z. physiol. Chem.*, 208: 33, 39 (1932). p. 75
Kimura, M., *Tôhoku J. Exptl. Med.*, 14: 275 (1929). p. 103
King, C. G., *see* Weber, H. H. R.
King, E. J., *Biochem. J.*, 25: 799 (1931). p. 76
ibid., 28: 476 (1934). p. 76
Kitagawa, R., *Tôhoku J. Exptl. Med.*, 24: 329 (1934). p. 87
Klemperer, G. and Scheurlen, E., *Z. klin. Med.*, 15: 370 (1889). p. 66, 80
Knudson, A., *J. Biol. Chem.*, 32: 337 (1917). p. 105
ibid., 45: 255 (1921). p. 105
Kohl, M. F. F., *J. Biol. Chem.*, 126: 709 (1938). p. 86
Kondo, K., *Biochem. Z.*, 26: 243; 27: 427 (1910). p. 76
Kosaka, T. and Lim, R. K. S., *Chinese J. Physiol.*, 4: 213 (1930); *C. A.*, 24: 4547 (1930). p. 80
Kruspe, H., *see* Ehrmann, R.
Kusumoto, C., *Biochem. Z.*, 14: 411 (1908). p. 105
Langworthy, C. F., *Ind. Eng. Chem.*, 15: 276 (1923). p. 60
Langworthy, C. F. and Holmes, A. D., *U. S. Dept. Agr., Bull.*, 310: 181, 188 (1915); *C. A.*, 10: 625 (1916) p. 59
Laroche, G., *see* Chauffard, A.
Lasnitski, A., *see* Rona, P.
Laszt, L., *see* Verzár, F.
Laszt, L. and Verzár, F., *Biochem. Z.*, 285: 356 (1936a). p. 94
ibid., 288: 351, 356 (1936b). p. 94
Leathes, J. B. and Meyer-Wedell, L., *J. Physiol.*, 38: xxxviii (1909). p. 100
Leathes, J. B. and Raper, H. S., "The Fats," New York, Longmans, Green and Co., 1925. p. 82
Lee, F. C., *Johns Hopkins Hosp. Bull.*, 33: 21 (1922). p. 102
Lehman, E. P., *J. Biol. Chem.*, 16: 495 (1914). p. 105
Lehmann, E., *see* Kaufmann, C.
Leites, S., *Arch. exptl. Path. Pharmakol.*, 129: 108 (1928). p. 106
Levites, S., *Z. physiol. Chem.*, 49: 273 (1906). p. 61, 66, 79
Lewis, H. B. and associates, *J. Biol. Chem.*, 56: 7 (1923); 67: 567 (1926). p. 75
L'Huillier, A., *see* Grigaut, A.
Lim, R. K. S., *see* Ivy, A. C., Kosaka, T.
Lim, R. K. S., Ivy, A. C. and McCarthy, J. E., *Quart. J. Exptl. Physiol.*, 15: 13 (1925). p. 80
Lockwood, B. C. and Chamberlin, H. G., *Arch. Internal Med.*, 31: 96 (1923). p. 79
Loeffler, K., *Z. physiol. Chem.*, 178: 186 (1928). p. 107
Loevenhart, A. S., *Am. J. Physiol.*, 6: 331 (1902). p. 77
ibid., 15: xxvii (1906). p. 63
J. Biol. Chem., 2: 427 (1907). p. 95, 96
See also Kastle, J. H.
Loevenhart, A. S. and Pierce, G., *J. Biol. Chem.*, 2: 397 (1907). p. 64
Loevenhart, A. S. and Souder, C. G., *J. Biol. Chem.*, 2: 415 (1907). p. 64, 84
Lombroso, U., *Arch. exptl. Path. Pharmakol.*, 56: 357 (1907). p. 60
ibid., 60: 99 (1908). p. 84
London, E. S., *Z. physiol. Chem.*, 50: 125 (1906). p. 66
Long, J. H. and Fenger, F., *J. Am. Chem. Soc.*, 39: 1278 (1917). p. 100
Longenecker, H. E. and Haley, D. E., *J. Am. Chem. Soc.*, 57: 2019 (1935). p. 71
Ludford, R. J., *see* Cramer, W.
Lyman, J. F., *J. Biol. Chem.*, 32: 7 (1917). p. 86
McCarthy, J. E., *see* Ivy, A. C., Lim, R. K. S.
McCay, C. M. and Paul, H., *J. Nutrition*, 15: 377 (1938). p. 60
McClure, C. W. and Huntsinger, M. E., *J. Biol. Chem.*, 76: 1 (1928). p. 100
McClure, C. W., Vincent, B. and Pratt, J. H., *J. Exptl. Med.*, 25: 381 (1917). p. 83
McClure, F. J. and Carr, R. H., *Am. J. Physiol.*, 74: 70 (1925). p. 62
McGuire, G., *see* Falk, K. G.
MacKay, E. M., *see* Barnes, R. H.

MacLean, I. S., see Smedley-MacLean, I.
Magendie, F., "Précis élémentaire de physiologie," Vol. 2, p 142, Paris, Mequignon-Marvis, 1825. p 66
Mancke, R., Z. physiol. Chem., 162, 238 (1927) p 108
Matlack, M. B., see Balls, A K
Mayer, A. and Morel, P., Bull soc. chim biol, 1: 189 (1919). p. 74
Mellanby, J., J. Physiol., 64: v, xxxiii (1927). p. 90
Memmen, F., see Willstätter, R.
Mendel, L. B. and Baumann, E. J., J. Biol. Chem, 22: 165 (1915). p. 81
Mendel, L. B. and Daniels, A. L., J. Biol. Chem., 13: 71 (1912). p. 90
Menschick, W. and Page, I. H., Z. physiol. Chem., 211: 246 (1932). p. 108
Meyer-Wedell, L., see Leathes, J. B
Michaelis, L., see Rona, P.
Michaelis, L. and Nakahara, Y., Z. Immunitäts., 36: 449 (1923) p 72
Miller, E. S., see Barnes, R H.
Milletti, F., see Traetta-Mosca, F
Mills, L. H., Arch. Internal Med., 7: 694 (1911). p. 103
Minkowski, O., Berlin. klin Wochschr, 27: 333 (1890). p. 83
Mjassnikow, A. L., Z. klin. Med, 103: 767 (1926). p. 106
Moore, B., Proc. Roy. Soc. London, 72: 134 (1903). p. 90, 92
Moore, B. and Rockwood, D. P., J. Physiol., 21: 58 (1897a); Proc. Roy. Soc. London, 60: 438 (1897b) p. 78
Morel, A. and Vellus, L., Bull. soc. chim. biol., 10: 478 (1928). p. 71
Morel, L. and Terroine, E.-F., Compt. rend. soc. biol., 67: 36 (1909). p. 68
J. physiol. path. gén, 14: 58 (1912). p. 75
Morel, P., see Mayer, A.
Mortimer, B., see Ivy, A C, Rony, H R.
Mottram, J. C., Cramer, W. and Drew, A. H., Brit. J. Exptl Path., 3: 179 (1922). p. 62
Mueller, J. H., J. Biol. Chem., 22: 1 (1915). p. 105
ibid., 25: 561 (1916a). p. 76
ibid., 27: 463 (1916b). p. 76, 105
Munk, I., Arch. path. Anat. (Virchow's), 122: 302 (1890). p 98
Ergeb. Physiol., 1: 296 (1902). p 103
Munk, I. and Rosenstein, A., Arch path Anat (Virchow's), 123, 230, 484 (1891). p 85, 86, 98, 101
Nakahara, Y, see Michaelis, L
Nedswedski, S., Arch ges. Physiol (Pflügers), 214: 337 (1926). p 101
Nikolaides, R, Arch. Physiol., 1899: 518. p. 98
Nikuni, J., J. Agr. Chem. Soc Japan, 7: 827 (1931); C A, 26 1323 (1932). p 108
Noguchi, H., Biochem. Z, 6, 185 (1907). p 74
Nomura, T., Tôhoku J. Exptl. Med, 4: 677 (1924) p 76
ibid., 12: 247 (1929) p. 103
See also Yamakawa, S.
Nothman, M. and Wendt, H., Arch. exptl. Path Pharmakol., 164: 266 (1932). p 70
Ogata, —, Arch. Physiol, 5: 515 (1881) p. 66
Ozaki, J, Biochem Z, 177: 156 (1926). p. 58
Page, I. H., see Menschick, W
Palmer, L. S., J Am. Chem Soc, 44: 1527 (1922) p 72
Papendieck, A, see Schumm, O.
Paul, H., see McCay, C M.
Pavlov, I. P., "The Work of the Digestive Glands," trans. by W H. Thompson, 2d English ed., London C Griffin, 1910. p. 68
Peretti, G., Arch. fisiol., 36: 113 (1936); C. A., 30: 7656 (1936). p 61
See also Artom, C
Perewoznikoff, A., Zentr. med Wiss., 54: 851 (1876). p. 85
Perlman, I., Ruben, S and Chaikoff, I. L., J Biol. Chem, 122: 169 (1937). p. 94
Petersen, W., see Jobling, J. W.
Pettenkofer, M v. and Voit, C., Z. Biol., 9: 1 (1873). p. 62
Pick, E. P., see Joannovics, G.
Pierce, G, see Loevenhart, A. S.
Porter, A. E., Biochem. J., 10: 523 (1916). p. 63, 76
Pottevin, H., Compt. rend. acad. sci., 136: 767 (1903). p. 68
Powell, M., J. Biol. Chem., 89: 547 (1930). p. 60
Pratt, J. H., Am. J. Physiol., 40: 145 (1916). p. 83
See also McClure, C. W.
Prewitt, P. V., Am. J. Physiol., 65: 287 (1923). p. 73
Pribram, H., Biochem. Z., 1: 413 (1906). p. 105
Rabbeno, A., Arch. sci. med., 38: 259 (1914). p. 103
Rachford, B. K., J. Physiol., 12: 72 (1891). p. 84
Radziejewski, S., Arch. path. Anat. (Virchow's), 43: 268 (1868); ibid., 56: 211 (1872). p. 85
Raper, H. S., J. Biol. Chem., 14: 117 (1913). p. 99, 102
See also Leathes, J. P.
Ravdin, I. S., see Riegel, C.
Ravenel, M. P., J. Med. Research, 10: 460 (1903). p. 90
Réale, L., see Artom, C.
Reed, C. C., Am. Rev. Tuberc., 7: 105 (1923). p. 74
Reiner, M., see Doubilet, H.
Reiser, R., J. Biol. Chem, 120: 625 (1937). p. 99
Rettie, T., see Smith, J. L.
Rewald, B., Biochem. Z., 198: 103 (1928). p. 104
Riegel, C., Elsom, K. O. and Ravdin, I. S., Am. J. Physiol., 112: 669 (1935). p. 90
Rockwood, D. P., see Moore, B.
Roger, H. and Binet., L., Compt. rend. soc. biol., 86: 79 (1922). p. 101
Rona, P. and Lasnitski, A., Biochem. Z., 152: 504 (1924). p. 73, 78
Rona, P. and Michaelis, L., Biochem. Z., 31: 345 (1911). p. 73
Rony, H. R., see Ivy, A. C.

Rony, H. R., Mortimer, B. and Ivy, A. C., *J. Biol. Chem.*, 96: 737 (1932). p. 98, 99
ibid., 102: 161 (1933). p 98
Rosenheim, O , *J Physiol.*, 40: xiv (1910). p. 69
Rosenstein, A., *see* Munk, I.
Ruben, S., *see* Perlman, I.
Rubner, M., *Z Biol.*, 15: 115 (1879). p 62
Sandberg, M. and Brand, E., *J. Biol. Chem* , 64: 59 (1925). p. 71
Sano, M., *Tôhoku. J. Exptl. Med.*, 4: 417 (1924). p. 106
Sarzana, G., *see* Artom, C.
Schäfer, E. A , *Intern. Monatsschr Anat Physiol.*, 2: 6 (1885). p. 87, 95, 96
Schantz, E J., Elvehjem, C A and Hart, E. B., *J. Biol. Chem.*, 122: 381 (1938). p. 106
Schenker, R , *Biochem. Z* , 120: 164 (1921) p. 71, 72
Scheurlen, E , *see* Klemperer, G.
Schiff, M , *Arch physiol. norm path* [5], 4: 699 (1892). p. 70
Schoenheimer, R , *Biochem. Z* , 147: 258 (1924). p 107
Schoenheimer, R , Behring, H v and Hummel, R., *Z. physiol. Chem.*, 192: 117 (1930). p. 108
Schoenheimer, R and Dam, H., *Z. physiol. Chem.*, 211: 241 (1932). p. 108
Scholl, E., *see* Fürth, O.
Schütz, J., *see* Fürth, O.
Schultz, J. H , *Biochem. Z* , 42: 255 (1912) p 76
Schumm, O. and Papendieck, A , *Z. physiol. Chem* , 131: 54 (1923). p. 86
Scott, T F M , *see* Holt, L. E.
Segré, E., *see* Artom, C.
Shope, R. E., *J. Biol. Chem* , 80: 125 (1928) p 76
Sicard, J. A., Fabre, R. and Forestier, G., *Bull soc chim biol* , 5: 413 (1923). p. 101
Sinclair, R G., *J Biol Chem.*, 82: 117 (1929). p. 92, 93, 102
ibid , 115: 211 (1936) p. 102
Sinclair, R G. and Smith, C., *J. Biol. Chem.*, 121: 361 (1937). p. 92
Skaer, W. F., *see* Greene, C. W.
Slowtzoff, B , *Beitr. chem. Physiol. Path.*, 7: 508 (1906). p. 104
Smedley-MacLean, I , *Biochem. J.*, 16: 370 (1922). p. 71
Smidt, H., *Arch klin. Chir.*, 125: 26 (1923). p. 80
Smith, C , *see* Sinclair, R G
Smith, J L. and Rettie, T., *Quart. J. Exptl. Physiol.*, 19: 51 (1928). p. 93
Sobotka, H. and Glick, D., *J Biol Chem.*, 105: 199 (1934a). p. 65
ibid , 105: 221 (1934b). p. 64, 65
Sorge, H., *see* Wieland, H.
Souder, C. G., *see* Loevenhart, A. S
Sperry, W M., *J. Biol Chem.*, 71: 351 (1927). p 98
ibid., 111: 467 (1935) p 76
Spiers, M. A., *see* Himwich, H. E.
Ssokoloff, N. A., *Deut. Arch klin. Med.*, 144: 202 (1924); *Z. ges exptl. Med.*, 46: 650 (1925). p. 105
Stade, W., *Beitr chem. Physiol. Path.*, 3: 291 (1903) p 67, 73
Starling, E. H., *see* Bayliss, W. M.
Steenbock, H , *see* Irwin, M. H.
Steenbock, H. and associates (Irwin, M H., Kemmerer, A. R , Templin, V. M. and Weber, J H.), Wisc Agr. Expt Sta., *Bull.*, 430: 127 (1935). p. 62
Sugiura, K., *see* Falk, K. G.
Süllmann, H. and Wilbrandt, W., *Biochem Z.*, 270: 52 (1934). p. 93, 104
Summers, W. S., *see* Greene, C. W.
Takata, H., *J Biochem.*, 18: 63 (1933) p. 76
Tangl, H. and Behrend, N., *Biochem. Z.*, 220: 234 (1930). p 100
Templin, V. M., *see* Irwin, M. H., Steenbock, H.
Terroine, E -F , "Contribution à la connaissance de la physiologie des substances grasses et lipoidiques," Paris, Masson et Cie, 1919 p. 61, 63, 66, 82, 84, 86
See also Kalaboukoff, L., Morel, L.
Thannhauser, S. J , *Deut. Arch. klin Med* , 141: 290 (1923). p. 76
Thiele, F H , *Biochem J.*, 7: 275, 287 (1913). p. 76
Tidwell, H. C., *see* Holt, L. E.
Tileston, W., *Arch. Internal Med* , 9: 525 (1912) p 90
Traetta-Mosca, F and Milletti, F , *Ann chim applicata*, 13: 270 (1923); *C A.*, 18: 2357 (1924). p 71
Tsuji, R , *Arch. ges. Physiol (Pflügers)*, 229: 344 (1932). p 74
Tucker, I. W., *see* Balls, A. K
Twort, C. C. and Twort, J. M , *J. Hygiene*, 33: 396 (1933). p. 89
Usawa, S., *J. Biochem.*, 15: 19 (1932) p. 75
Vellus, L., *see* Morel, A.
Verzár, F., *Ergeb. Physiol.*, 32: 391 (1931). p. 96
Nutrition Abstracts & Rev , 2: 441 (1933) p 93
See also Laszt, L.
Verzar, F and Laszt, L., *Biochem Z* , 270: 24 (1934a). p. 93
ibid., 270: 35 (1934b). p. 94
Vickery, J. R., *J. Council Sci Ind. Research*, 9: 107 (1936) p. 71
Vincent, B., *see* McClure, C. W.
Voit, C., *see* Pettenkofer, M. v.
Volhard, F., *Münch. med. Wochschr.*, 47: 141; 194 (1900). p. 66
Z. klin. Med , 42: 414, 397 (1901). p. 66, 67
Wacker, L and Hueck, W., *Biochem. Z.*, 100: 84 (1919). p 107
Waldschmidt-Leitz, E , *see* Willstätter, R
Walther, P. v., *Arch. Physiol. (Du Bois-Reymond's)*, 1890: 329 p 92, 99, 101
Wartenberg, H., *see* Connstein, W.
Weber, H. H. R and King, C G., *J. Biol. Chem.*, 108: 131 (1935). p 65
Weber, J. H., *see* Steenbock, H.
Weinstein, S. S. and Wynne, A. M., *J. Biol. Chem.*, 112: 641, 649 (1936). p 61
Weiss, O., *Arch. ges Physiol. (Pflügers)*, 144: 540 (1912). p. 80

Wendt, H., *see* Nothman, M.
Whipple, G. H., *Johns Hopkins Hosp. Bull.*, 24: 357 (1913). p. 78
Wick, A. N., *see* Barnes, R. H.
Wieland, H. and Sorge, H., *Z. physiol. Chem.*, 97: 1 (1916). p. 107
Wilbrandt, W., *see* Süllmann, H.
Willstätter, R., *Sitzber. math. naturw. Abt. bayer Akad. Wiss. München*, 1924: 1. p. 63
Willstätter, R. and Memmen, F., *Z. physiol. Chem.*, 133: 247 (1924a). p. 67
ibid., 138: 216 (1924b). p. 63, 64, 69, 76
Willstätter, R. and Waldschmidt-Leitz, E., *Z. physiol. Chem.*, 134: 161 (1924). p. 71
Wimmer, E. J., *see* Hughes, R. H.
Wintersseel, W., *see* Bürger, M.
Wohlgemuth, J., *Z. physiol. Chem.*, 44: 540 (1905). p. 66
Biochem. Z., 39: 302 (1912). p. 68
Wolvekamp, H. P. and Griffioen, K., *Z. physiol. Chem.*, 223: 36 (1934). p. 64
Wotton, R. M. and Zwemer, R. L., *Anat. Record*, 75: 493 (1939). p. 88
Wwedensky, N. and Dobrowitzky, P. J., *Biochem. Z.*, 188: 448 (1927). p. 73
Wynne, A. M., *see* Weinstein, S. S.
Yamakawa, S., Nomura, T. and Fujinaga, I., *Tôhoku J. Exptl. Med.*, 14: 265 (1929). p. 90
Yosinaga, T., *J. Biochem.*, 24: 21 (1936). p. 76
Ziegler, K., *Z. ges. exptl. Med.*, 24: 242 (1921). p. 103
Zinsser, A., *Beitr. chem. Physiol. Path.*, 7: 31 (1906). p. 66
Zucker, T. F., *Proc. Soc. Exptl. Biol. Med.*, 17: 89 (1920). p. 101, 103
Zwemer, R. L., *see* Wotton, R. M.

Chapter III

Lipids of Blood

Introduction

Blood as a tissue

The blood is a fluid tissue consisting of cells or corpuscles suspended in serum. Its main function is that of carrier of raw materials and cellular products to and from the fixed tissues. In performing this function, the cells and serum may act together or independently, the transported material distributing itself in general according to the known laws of diffusion, subject, however, to a variety of little-understood selective activities of which a good illustration is the fact that potassium is mainly in the cells and sodium in the extracellular fluids. The blood proteins and other larger molecular substances exhibit the characteristic ability to form more or less loose combinations with the materials with which they come into contact. These combinations often affect their separation from other blood constituents in analytical procedures. The corpuscles behave like other tissue cells in tending to preserve a constant composition which is characteristic of this particular group of cells and relatively independent of species, while the composition of the serum tends to be variable and is characteristic of the species. The blood differs from the fixed or solid tissues in having a larger proportion of tissue fluid. A further difference from other tissues lies in the fact that in many animals a large proportion of the cells or corpuscles have no nuclei, which undoubtedly limits their "vital" activity. The blood is contained in vessels, the arteries, veins and capillaries, of which the capillaries alone are permeable to the large molecular and large particulate substances, behaving in this respect like membranes of a definite pore size which are, however, different in different locations and under different conditions. The surfaces with which the blood has contact consist of living cells which may be able to do work in modifying the passage of substances through them or near them or even produce chemical changes in the substances themselves. All these factors influence the composition of the blood and must be taken into account in considering the behavior of the substances carried in the blood. Diseased states would be expected to influence blood composition according to their nature and extent and the extent of the efforts to counteract

them. The case of the lipids, with the possible exception of some of the phospholipids, is further complicated by the fact that they are large molecular compounds insoluble in water, and are carried in the blood as colloidal aggregates, so that their passage in and out of the blood stream and their behavior in it involve additional factors resulting from their insolubility. For example, there appears to be no free interchange of lipid between plasma and corpuscles such as is the case with dextrose or urea, although there may be a slow exchange. Biological variation, *i.e.*, differences in value and in reaction to experimental procedure between different animals of the same species, is often large in the case of the lipids and must be taken into account both in establishing normal levels and in determining the effect of abnormal states or experimental procedures.

Nature of the fatty substances of blood

The lipids of both plasma and corpuscles in normal animals which are neither fasting nor actively absorbing from the intestine consist of:

(a) neutral fat in small amounts;
(b) phospholipid: lecithin and cephalin in relatively large amounts, and sphingomyelin in varying amounts; in plasma, lecithin predominates, while in the corpuscles cephalin and sphingomyelin make up most of the phospholipid;
(c) cholesterol in amounts of the same order of magnitude as phospholipid: in corpuscles almost entirely in the free form, in plasma, 60-70 per cent in ester combination with fatty acids;
(d) other "unsaponifiable" of little known nature in about the same amount as cholesterol;
(e) fatty acids in other combinations than those mentioned, and possibly free fatty acids in small quantities.

The "unsaponifiable" matter of beef and dog plasma has been examined by Anderson (1926), who found that in beef plasma it consisted almost entirely of crystalline cholesterol with no evidence of the presence of phytosterols, although the animals had received only plant sterols in their food (see also Schoenheimer, 1929). The "unsaponifiable" from dog plasma was a much more complex mixture. It was impossible to prepare pure cholesterol from it by direct crystallization and therefore it was treated with digitonin. The material precipitated by digitonin consisted of about three-fourths cholesterol and less than one-fourth of a yellow, waxy substance which contained very little cholesterol. About 18 per cent of the original unsaponifiable material was not precipitated by digitonin, although it gave the Liebermann-Burchard reaction and was there-

fore probably closely related to cholesterol. In all, about 37 per cent of the "unsaponifiable" consisted of non-crystalline material, the nature of which was unknown. Anderson's results show that the unsaponifiable substance of blood plasma is often a complex mixture of a variety of substances including different sterols and sterol products, and explain the difficulty found in arriving at a satisfactory method for their quantitative determination, and in particular, for the determination of cholesterol.

The fatty acids in the phospholipids of plasma, as ordinarily found, are not highly unsaturated [iodine number for fatty acids of beef plasma phospholipid, 71 (Bloor, 1923, 1924, 1925; Channon and Collinson, 1929); somewhat higher for pig plasma, 80; and dog plasma, 89 (Bloor, 1923, 1924, 1925)]. The apparently free fatty acids have the same iodine value as the phospholipid fatty acids and probably of the fat, although certain information on the latter point is not available (Bloor, 1923, 1924, 1925). As found by Sinclair (1936), the fatty acids of the blood phospholipids represent to a considerable extent the fatty acids of the fat most recently absorbed, *i.e.*, they represent the absorbed fat in transport. The phospholipid fatty acids consist ordinarily of two to three parts of liquid acid (unsaturated) to one part of solid saturated acid (Snider and Bloor, 1933; Sinclair, 1935).

The solid fatty acids in combination with the phospholipid consist mainly of palmitic acid, and the unsaturated acids are mainly oleic and linoleic. Channon and Collinson (1929) found that the fatty acids of the acetone-soluble fraction of beef blood were arachidonic, linoleic, stearic, and palmitic, and that 55 per cent of the fatty acids were in combination as cholesterol ester, and 25 per cent as fat. Schaible (1932), as the result of a study of the plasma lipids of lactating and non-lactating cows, found that the fatty acids of the plasma lecithin are of a much lower degree of unsaturation than those of cholesterol esters, while those of the neutral fat are intermediate. He thinks, therefore, that cholesterol esters play a part in fat transport. Lipids were higher in the blood of lactating than of non-lactating cows, but the nature of the contained fatty acids was similar. Bloor, Blake and Bullen (1938) found in normal human plasma that the degree of unsaturation of the fatty acids progressively increased from the neutral fat (iodine number 102) to the phospholipid (iodine number 125), and was highest in the cholesterol esters (iodine number 158).

As may be seen from the data given in Tables 6 and 7 (pages 123 and 124), the percentage amounts of the constituents of the plasma or serum vary greatly in different species and are often very low in herbiv-

orous animals. The composition of corpuscles tends to be constant for all species.

Blood lipid equilibrium: the postabsorptive state

For the study of the composition of the blood, and its constancy or variability under normal or abnormal conditions, it is necessary to have a point of reference at which the composition is at its maximum constancy. This would be the time when it is least influenced by the substances which it transported, and the ideal point of reference would be the time when it is neither taking up nor giving out the material used by the tissues with which it comes in contact. It would be a time when no food material is coming in from the intestine and yet before the fasting state had started a mobilization from the stores—a state of affairs which probably never exists. This hypothetical time has been given the name of postabsorptive time or state. The actual time chosen is generally arbitrary. Terroine (1914b), in his study of the lipid composition of blood, chose for the time of sampling in dogs 36 hours after the last meal, and his results show that a good degree of constancy in composition was obtained at that period. However, this is probably too long a period, especially for the smaller laboratory animals which would undoubtedly be drawing on their reserves by that time. It is also inconveniently long for clinical studies on human beings. The time most frequently made use of is 16 to 18 hours after the last meal, and is chosen largely as a matter of convenience to fit the ordinary laboratory day. Shorter periods have been recommended for different food and tissue constituents with apparently satisfactory results, and the fact of the matter seems to be that the disturbance in blood composition produced by the inflow of food from either the digestive tract or the stores is compensated for fairly rapidly, so that a sufficiently good point of reference for analytical studies is obtainable over a wide period of time after the last meal.

How long does the influence of a fatty meal on the blood lipids last, and how soon is the postabsorptive steady state resumed? Most workers are agreed that the peak of the blood fat curve after a relatively large meal of fat comes about the fourth to the sixth hour, and that after that the fall is fairly rapid. How long may suspended fat stay in the blood? As indirect evidence on this point may be mentioned the experiments of Bondi and Neumann (1910), who found that inorganic suspensions, such as collargol, disappeared in about one-half hour, as was also the case with emulsions of lanolin, cholesterol, lecithin, butter, and olive oil. Injected colored fat and iodized fat were found in the liver, bone marrow, spleen, and muscles, in about the order named. Schott (1913) injected egg yolk, which contains about one-third of its weight of lipid in a very finely

divided form, and found that the fat particles had disappeared in less than one-half hour. Bloor (1914) injected casein emulsions of fat, consisting of particles 2 to 5 μ in diameter, and could detect none of the fat in the blood immediately after, although enough had been injected to double the blood fat if it had remained in the circulation. Then egg yolk from day-old eggs was diluted with an equal volume of salt solution and filtered, then injected. The particles in this case were 1 to 2 μ in diameter. The effect here was quite marked, the blood fat rising to more than double the normal value but soon falling, until at the fourth hour it was down to the normal level. It was interesting to note that in a dog which had been fasted for eight days, the rise in blood fat was greater and the fall much more abrupt than in the fed animal. In these experiments, most of the injected fat (over 80 per cent) could be accounted for in the blood in the first sample taken as soon as possible after the injection was completed. From these results, the general assumption that the effect of fat absorption is over by the fourteenth hour seems well on the safe side. The general practice with humans is to take the sample before breakfast, that is, 12 to 16 hours after the last meal, which would seem to be a suitable time as regards the fat, although whether the other lipids have come to an equilibrium at this time is not known.

How long does this steady state last in the fasting individual? How long is it before the mobilization of fat from the stores begins to show its effect on the blood lipids? Students of metabolism are familiar with the fact that the animal organism in starvation uses its carbohydrate stores first, but these are not extensive, and it is generally believed that their effect has practically entirely disappeared by the third day of fasting; also, that the available loosely stored protein is gone by that time. Probably considerably before that time, the fat of the stores is being mobilized, although perhaps at a rate that would have little if any effect on the blood lipid levels. In the lack of definite information, it may be assumed that, for the larger animals at least, the blood lipids would be at equilibrium in the period of from 14 to 36 hours after the last meal; in small animals, such as the rat or mouse, the time would probably be considerably shorter.

Lipid level in the postabsorptive state

Data on the lipids of the blood in the postabsorptive state have been obtained by many workers. Terroine, working at 36 hours postabsorptive in dogs and taking the further precautions of a standard diet for a considerable time and avoidance of struggling in drawing the blood, which tends to produce fat mobilization, found that although the content of the blood in total fatty acids and cholesterol might vary 100 per cent from

animal to animal, the values for individuals varied little from time to time over several months. For total lipids, the greatest variation found in any individual animal from time to time was 32 per cent, but the average variation from the average in all the individual animals examined was only 5.4 per cent. The cholesterol content showed similar differences from animal to animal and similar constancy in individuals. The same was true of the relation between cholesterol and fatty acids (*cholesterol/fatty acids*).

These results concerning the fatty constituents of the blood support the conception that it is a tissue and also indicate that, as Mayer and Schaeffer (1913) have shown for other tissues, it has a fairly constant composition in individuals, as regards both total lipid content and the relation between the lipid constituents. Results of later workers support, in general, these findings and conceptions, although they are not unanimous. Thus, Bloor (1914), as the result of complete lipid examination of a considerable number of human blood samples, found a like constancy of relation between the constituents, and was led to the belief that there was in the postabsorptive state an efficient regulation of the lipid constituents of the blood. Bang (1918a) arrived at the same conclusions about fatty acids and cholesterol, and stated his belief that the content of these substances in blood was regulated within narrow limits.

Factors affecting the lipid level

The level of the fatty substances in the blood might be expected to be influenced by individual variations in the nature and behavior of the blood as a tissue, by the rate of inflow of fatty material from the gastrointestinal tract or from the lipid stores, by the rate of outflow to the tissues, and possibly by excretion.

Adjustment between lipids. A relatively constant relationship between cholesterol and phospholipid has been found and characterized as *cholosterol/lipid* phosphorus by Mayer and Schaeffer. Whether the constant relationship found between these lipids in the postabsorptive condition (which is the time when the amount of neutral fat is small) holds when fat is added to the system, as during fat absorption or mobilization, is a matter of dispute, different workers having obtained various results. Some have found that the addition of fat to the blood results in an increased blood cholesterol (Chauffard, Laroche and Grigaut, 1920), and increase of phospholipid during fat absorption has been reported by most of those who have looked for it (Bloor, 1916a; Bang, 1918b; Knudson, 1917). Increase of blood cholesterol during the absorption of fat has not been found by all workers (Bloor, 1916a; Knudson, 1917; Bang, 1918b). In view of the inconsistency of these findings, it must be said

that although the adjustment of the lipids to each other is perhaps characteristic of the blood as a transport medium for fat, animals vary a good deal in the extent of response.

Protein combinations. The combination of fatty substances in process of transport with blood constituents, for example, proteins, chloride, and carbon dioxide, might be expected to be a factor in determining the blood lipid levels, and these combinations have been discussed at length later in the chapter (p. 185 ff.).

Input and removal mechanism for lipids. The principle of inertia or lag may be expected to play a part in all processes of metabolic interchange. It has been very well shown for sugar (Gilbert and co-workers, 1926), that outflow does not start at once in full swing when sugar reaches the blood from the intestine, but that the incoming material is allowed to accumulate until a certain level is reached before the removal mechanism is stimulated to act. Once set in motion, it continues to act until a rather low level of blood sugar is reached; then it ceases until again stimulated by a new high level, and so on through the period of absorption. In each case, the stimulating level and the point at which the outflow ceases both become lower, until at the end the blood sugar is often below the initial normal level. These facts indicate that the sensitivity to stimulation of the removal mechanism increases with use. The effect of lack of practice with regard to carbohydrates is shown especially well in fasting animals (Benedict, Osterberg and Neuwirth, 1918; du Vigneaud and Karr, 1925) in which the sugar in the blood reaches abnormally high values before removal begins. Similar behavior has been found for the fat removal mechanism. Thus Rony and Ching (1930) have found that the state of nutrition of the animal affects the response of the blood to a standard dose of fat, and to get a standard reaction, it was necessary to fast the animal for 7 to 14 days. Insulin reduced or inhibited the lipemia, as they believed, because the promotion of carbohydrate fixation favored the fixation of fat by the tissues. The variation in sensitivity of the removal mechanism may be extreme, as shown by the fact that marked lipemia may sometimes clear up when a high-fat diet is fed (Blatherwick, 1921; Blix, 1926). No sharp changes in lipid level can be noted, such as occur in blood sugar during sugar absorption, a fact which might be expected because of the numerous shifts in combination undergone by the fatty acids during absorption. The mode of action of the mechanism for the removal of fat from the blood is unknown, as is also the reverse—the mobilization of fat from the depots into the blood.

Apparent differences due to method. While an unsuitable time for sampling may account for some of the differences in reported values of blood lipids in the postabsorptive state, the methods used are probably

also responsible. Thus, the method which in the past was accepted as standard by most workers, the Kumagawa-Suto method for total fatty acids, suffers from the defect that as a result of exposure of the material to air for considerable periods at high temperature in alkaline solution, some of the fatty acids are so changed by oxidation that they are no longer soluble in petroleum ether and so are included in the unsaponifiable matter rather than in the total fatty acids. The Windaus method for cholesterol—precipitation as the digitonide, regarded as the standard method for cholesterol—has been shown (Anderson, 1926) to precipitate other constituents of blood as well as cholesterol. Lecithin or phospholipid is almost always measured by its phosphoric acid content, which is about one-eighth of the weight of the molecule of lecithin or cephalin; and while it is certain that all the important phospholipids in tissues are neither lecithin nor cephalin, it is probable that this assumption is nearly correct. On the other hand, the materials measured as lipid phosphorus are the ether- or alcohol-soluble phosphoric acid compounds, and it has been shown (Le Breton, 1921) that in this fraction, phosphorus compounds other than lecithin and cephalin may be present to the extent of up to 20 per cent. Still another disturbing factor applying especially to the analysis of the lipids is the factor of intersolubility. For example, both phospholipid and cholesterol are soluble in fat, and the universal presence of fat adds a complication to the isolation and measurement of these substances.

Normal Basal Levels of Blood Lipids

Average Values

Data on different species

The amount of work done on the lipid constituents of blood is large and the literature correspondingly extensive. Much of this work has been done on human blood but rather more on that of the lower animals, and the results are so interrelated that they may be considered more or less together. The values found are often contradictory and confusing because of the variety of methods used.

All analytical work on changes in blood composition is based on the comparison with normal values and consequently the determination of normal values is fundamental. The earliest values for blood lipids by reasonably modern methods are those furnished by Abderhalden (1911, p. 554) and quoted in Table 6. It is not certain from this table that much attention was paid to the time of sampling with reference to the post-absorptive state. However, the natural range in values is so wide that unless samples are taken during actual fat absorption the figures will not

Table 6. Blood Lipid Values (Abderhalden, 1911).
(Parts per Thousand Parts by Weight)

	Water			Cholesterol			Lecithin			Fatty Acids			Fat	
	Blood	Serum	Corp.	Blood	Serum	Corp.	Blood	Serum	Corp.	Blood	Serum	Corp.	Blood	Serum
Cow	808.9	913.6	591.9	1.935	1.238	3.379	2.349	1.675	3.748	.....	.....	.....	0.567	0.926
Bull	814.8	913.4	618.6	1.209	1.901	1.824	2.197	1.869	2.850	0.495	0.743	.....	2.363	3.542
Sheep I	821.7	917.4	604.8	1.332	0.879	2.360	2.220	1.709	3.379	0.488	0.710	.....	0 937	1.352
Sheep II	824.6	916.8	627.8	2.038	1.309	3 593	2 417	1.599	4.163	0 490	0.721	.....	0.864	1.262
Goat	803.9	907.7	608.7	1.299	1.070	1.730	2.466	1.727	3.856	0.395	0.611	.....	0.535	0.624
Horse I	749.0	902.1	613.2	0.346	0.298	0.388	2.913	1.720	3.973	.....	.....	.....	0.611	1.300
Horse II	795.0	915.1	613.2	0.576	0.521	0.661	2.982	1.746	4.855	0.387	0 604	0.060	0.534	0.834
Pig	790.6	917.6	625.6	0.444	0.409	0.489	2.309	1.426	3.456	0.775	0.794	0.062	1.095	1.956
Rabbit	816.9	925.6	633.5	0.611	0.547	0.720	2.827	1.760	4.627	0.507	0.809	.....	0.734	1.193
Dog I	810.1	924.0	644.3	1.298	0.709	2.155	2.052	1.699	2.568	0.759	1.221	0.088	0.631	1.051
Dog II	792.0	923.0	627.2	0.922	0 658	1.255	1.994	1.755	2 296	0.684	1.254	.....	0.914	1.642
Cat	795.5	926.9	624.2	0.895	0.600	1.281	2.325	1.716	3.119	0.280	0.499	.....	0.373	0.788

be far off. Abderhalden's data are fairly representative. Wide variations are to be noted in blood lipid values of different species, and lesser, but still extensive, variations between individuals of the same species. More recent analyses are taken from the work of Mayer and Schaeffer (1913), who used the Kumagawa-Suto method for total fatty acid, the Windaus method for cholesterol, and determined phospholipid from ether-soluble phosphorus. Extracts from their results are given in Table 7.

Table 7. Blood Lipid Values (Mayer and Schaeffer, 1913).
(Dry Weight in Mg. per 100 Grams)
(Moist Weight in Mg. per 100 Cc.)

	Total Fatty Acid			Phospholipid			Cholesterol		
	Serum		Corp.	Serum		Corp	Serum		Corp.
	Dry	Moist	Dry	Dry*	Moist†	Dry*	Dry	Moist	Dry
Guinea pig	1679	102	822	253	15	902	383	23	336
Rabbit	2472	174	868	704	45	1573	515	41	325
Sheep	2058	176	907	704	48	1045	909	77	405
Horse	3092	279	862	1463	130	836	909	83	353
Cow	2280	171	869	1100	93	726	1182	83	359
Hen	7469	498	1247	3685	240	1364	1700	116	373
Dog	4716	406	1082	3245	275	957	1371	117	332
Pig	2930	271	951	803	68	1199	1280	124	376
Eel	22370	1957		10241	883		6836	163	
Murena	9006			308			2411		
Roussette	2249						479		

* Phospholipid obtained by multiplying P_2O_5 values by 11.
† Phospholipid obtained by multiplying P by 25.

Mayer and Schaeffer call attention to the very wide variations in serum lipid values in the different animals as compared with the relatively much narrower variations in corpuscle values. They believe that corpuscles are like other tissue cells in having a relatively constant composition, regardless of species. They note also the fact that the different constituents, especially phospholipid and cholesterol, vary together, preserving a more or less constant relation to one another.

These gross or macromethods have the obvious disadvantage of requiring so much blood for each determination that the health of the animal may be impaired and serial determinations would be impossible except in the large animals. As a consequence, micromethods have been developed requiring only a few cubic centimeters of blood for an analysis. Values by these methods are more abundant. Horiuchi (1920a) made a study of the lipids of plasma and corpuscles of rabbits, using the nephelometric method for fatty acids and the colorimetric method for cholesterol. Results on 19 rabbits are given in Table 8 and compared with the values obtained on dogs and humans by the same methods.

In this table is shown very clearly the fact, noted by Mayer and Schaeffer, of the closely similar values for the corpuscles in the different species as compared with the wide variations in plasma values. Group A of the rabbits was on a low fat diet (carrots), Group B on a relatively

Table 8. Blood Lipid Constituents of Man, Dogs, and Rabbits.
(Grams per 100 Cc.)

	Total Fatty Acids			Phospholipid			Cholesterol		
	Blood	Plasma	Corp.	Blood	Plasma	Corp.	Blood	Plasma	Corp.
Rabbit*									
Group A	0.28	0.22	0.36	0.21	0.11	0.40	0.10	0.06	0.16
Group B	0.34	0.30	0.42	0.21	0.11	0.38	0.11	0.07	0.18
Dog†	0.52			0.33			0.17		
Men‡	0.36	0.38	0.36	0.30	0.22	0.40	0.21	0.22	0.19
Women‡	0.36	0.40	0.29	0.29	0.19	0.44	0.23	0.24	0.21

	T. F. A./Lecithin			Lecithin/Cholesterol		
	Blood	Plasma	Corp.	Blood	Plasma	Corp.
Rabbit*						
Group A	1.34	2.19	0.91	2.10	1.82	2.52
Group B	1.70	2.93	1.13	1.92	1.53	2.22
Dog†	1.58			1.90		
Men‡	1.20	1.68	0.89	1.44	0.93	2.33
Women‡	1.31	2.15	0.69	1.29	0.82	2.14

* Values of Horiuchi (1920a) · 19 rabbits in group A (low fat diet); 10 rabbits in Group B (high fat diet).
† Values of Bloor (1915): calculated from dogs 10, 21, 23, and 24.
‡ Average values in humans (Bloor, 1916c).

high fat diet (sunflower seeds). The only notable effect of the diets on the blood lipids in this work is to be seen in the total fatty acids, which average higher throughout.

Blood phospholipid was determined by Bloor (1921a) and by Sundstroem and Bloor (1920), using the nephelometric method. Bloor found, in 25 rabbits, variations in plasma phospholipid from 40 to 200, averaging 92 mg. per 100 cc.; for corpuscles from 300 to 880, averaging 540 mg. per 100 cc. Average values in various animals are given in Table 9. The relative constancy of the corpuscle values and the variability of the plasma values in the different species are apparent.

Table 9. Blood Phospholipid (Bloor, 1921a).
(Mg. per 100 Cc.)

Animal	No. of Samples	Plasma	Corpuscles
Human	21	192	448
Dog	5	240	440
Beef	3	144	400
Calf	4	72	400
Cat	3	144	600
Rabbit	19	92	544
Hen	2	...	496

The variability of the lipids in the blood plasma of various common animals is also brought out in a study made by gravimetric methods (500 cc. of plasma digested with strong alkali, the acidified solution extracted, and the extracted material separated into fatty acids and unsaponifiable, and weighed). The average values and ranges obtained are given in Table 10.

The constancy of the lipids in the corpuscles has been emphasized by several workers, some of whom have been already mentioned. In

Table 10. Lipid Composition of Blood Plasma by Gravimetric Methods (Bloor, 1923).
(Mg. per 100 Cc.)

Animal	No. of Samples	Total Fatty Acid Av.	Total Fatty Acid Range	Unsaponifiable Av.	Unsaponifiable Range
Pig	13	184	97–300	106	68–130
Beef	16	154	102–206	114	67–184
Sheep	18	140	94–179	78	51–102
Dog	6	307	271–357	160	123–209

addition, the experiments of Iwatsuru (1924) led him to the conclusion that the lipid content of the corpuscles of different mammals is nearly the same, consisting of cholesterol, 215 mg., and total lipid, 580 mg. per 100 cc. of corpuscles. Iscovesco (1912) was impressed by the similarity in lipid content of the corpuscles of different animals. His values are given in Table 11.

Table 11. Lipid Values of Corpuscles and Serum (Iscovesco, 1912).
(Mg. per 100 Cc.)

	Fatty Acids Serum	Fatty Acids Corp.	Cholesterol Serum	Cholesterol Corp	Total Lipid Serum	Total Lipid Corp
Human	455	293	101	96	558	388
Horse	334	308	91	83	425	391
Rabbit	312	326	111	108	424	433

Constancy in corpuscle values is also found in cold-blooded animals; Tamura (1932) gives the cholesterol content of their corpuscles as 78 mg. per cent and the total fat as 259 mg. per cent, which are constant as compared to the variable serum content. The percentage of total cholesterol present in the ester form in the serum was 42 to 64, which is not far from the value in warm-blooded animals. De Waele (1929) found that the cholesterol in the blood of a mussel (*Anodonta Cygnoea*) was of the same nature as in higher animals, and that the amount was 0.45 mg. per 100 cc., of which 0.36 mg. was in the plasma and 0.09 mg. per 100 cc. in the corpuscles. These values are about 1 per cent of those found in higher animals.

Such small differences in corpuscle values as are found between different species may, according to Mayer and Schaeffer, be referred to differences in the amount of water present in the corpuscles, a fact which leads them to the belief that the concentration of total fatty acids and phospholipid in terms of the *dry weight* is the same for all species, and that the corpuscles are then in the same class as cells of other tissues. As noted above, the few analyses which have been published since their work appeared bear out their conclusions and lead to the belief that when it is desired to study the blood as a transporting medium for the lipids, plasma or serum only should be used, since the corpuscles take little part in the changes in the plasma. On the other hand, certain data on changes in the lipid content of the corpuscles indicate that we should not be too

hasty in reaching such a conclusion. For example, it has been found that the fat and phospholipid values of the corpuscles may increase during fat absorption (Munk, 1902; Bloor, 1915; Knudson, 1917; Bodansky, 1931). Terroine (1919, p. 358) has presented data on two dogs to show that the content of total fatty acids and cholesterol of the corpuscles may increase greatly as the result of abundant and repeated bleedings. Though it is also generally stated that the cholesterol of the corpuscles is all in the free state, Pfeiffer (1930) found that the corpuscles were not always free of ester: the ester content was lowest in winter, but in summer the total cholesterol and percentage as ester increased. Knudson (1917) found an increase in cholesterol esters in the corpuscles during fat absorption, as did also Bodansky (1931).

As is apparent, most workers have confined their attention to the more usual analyses of whole blood, serum, or total corpuscles. However, a few more specific analyses are available. Brun (1939) made extensive studies of the cholesterol of red blood corpuscles, both normal and pathological. In 58 normals he found an average value of 139 mg. per cent, with a range of 123 to 155 per cent all free. The content was constant for the individual, and feeding 4 grams of cholesterol in olive oil produced only insignificant changes. Sixty-two cancer patients averaged 155 mg. per cent with a range of 134-198 mg. per cent. Jaundice caused increases up to 70 per cent and parallel with the severity. Thannhauser, Setz and Benotti (1938) found in red blood corpuscles *diamino : monoamino phospholipids* as *1 : 2*. The sphingomyelin contained mainly lignoceric acid. Boyd (1936) gives a series of analyses of red blood cells showing the effect of oxalate as an anticoagulant in increasing the apparent lipid content by dehydration of the red cells (Table 12).

Table 12. Comparison of Lipid Values in Red Blood Cells from Defibrinated and Oxalated Blood (Boyd, 1936).
(Mg. per 100 Cc. of Red Blood Cells)

Case No.	Blood	Red Blood Cells (vol. %)	Total Fatty Acids	Total Cholesterol	Free Cholesterol	Phospholipid
1	Defibrinated	44.3	268	178	128	351
	Oxalated	40.0	280	190	140	367
2	Defibrinated	49.1	251	144	120	318
	Oxalated	38.2	313	150	130	395
3	Defibrinated	46.0	247	122	116	244
	Oxalated	39.1	306	145	134	377
4	Defibrinated	45.8	267	120	99	270
	Oxalated	43.3	259	132	105	286
5	Defibrinated	47.7	267	122	114	294
	Oxalated	42.8	248	127	115	316

The lipid content of rabbit white blood cells has been studied by Boyd and Stevenson (1937), who found for total lipid, 1860 ± 475; for neutral fat, 552 ± 177; for phospholipid, 864 ± 295; for free cholesterol, 304

± 94; and for bound cholesterol, 84 ± 45 (total cholesterol, 388 ± 111). These values are slightly higher than those found by Boyd (1933b) in women, but are on about the same level. In general, the composition resembles that of tissue cells rather than of plasma. The cholesterol ester content is lower than in plasma but higher than in most tissues.

Studies have also been made on the stromata of red blood corpuscles. Bürger and Beumer (1913) report that the lipids of the stromata of sheep erythrocytes consist of one-third cholesterol, one-third sphingomyelin, and one-third other phospholipid. In human corpuscles, most of the phospholipid is sphingomyelin and is water-soluble. They find the stroma to be 2.0 to 2.5 per cent of the weight of the whole corpuscle. Macy and associates (1938) found that the dried stroma of sheep, cow, and horse cells contained 22 per cent of lipid; man, 11 per cent; birds, 3 per cent; of the lipid, about 60 per cent was phospholipid, 30 per cent was free cholesterol, and 10 per cent fat. Stroma contains 50 to 75 per cent of the phospholipid as cephalin; plasma contains much less—20 per cent.

Blood platelets have been analyzed by Erickson, Williams, Avrin and Lee (1939). They found lipid values in per cent of dry substance for normal adults as follows: total lipid 16, phospholipid 12 (68 per cent cephalin), free cholesterol 2, cholesterol ester 1, and neutral fat 1.

Lipid constants

As the result of extensive studies of the lipid values of various tissues, including blood, Mayer and Schaeffer found constancies in amount and in relationships between constituents which appeared to be characteristic of the tissue. These relationships are: *total fatty acids/phosphorus*, *cholesterol/total fatty acids* (lipemic coefficient or constant), and the lipemic index (*total fatty acids plus cholesterol*). For example, in blood corpuscles, they found that the relation, *total fatty acids/total lipid phosphorus*, is "remarkably constant" at 23 for all species except the rabbit. Their lipemic constant they believe to be important in relation to the water content of cells.

Terroine (1919, p. 331) found for the relation, *cholesterol/total fatty acids* × *100*, in whole blood of fourteen dogs extreme variations of from 23 to 50, with an average value of 36 and an average variation from the average of 17 per cent with, in general, much smaller variations in the same animals. He had found previously (1914b) that this relation is preserved during fat absorption, the cholesterol of the blood rising parallel with the fat. The data of others have not always supported him in this respect, although cholesterol has often been found to increase with the fat during fat absorption. He concluded (1914b) that the total lipid value of blood (the lipemic index) is a constant for the individual; also

that the relation, *cholesterol/fatty acids* (lipemic constant), is constant for the individual, and that the two values, lipemic index and lipemic constant, define the animal. Thus, one animal will have 1.8 for total lipid and 26 for the constant; another animal will have 1.8 and 31; and another 1.9 and 29. Their determination in a given individual gives a basis for following changes due to physiological states or experimental modifications.

Bloor (1916b) believed that he had found for normal human blood constant relationships of lecithin to cholesterol and that the same values of the ratio held for pathological samples. Horiuchi (1920a) found that for rabbits the ratios *total fatty acids/lecithin* and *lecithin/cholesterol* held very closely to the racial and individual average used. Grigaut and Yovanovitch (1924) found that cholesterol and lecithin preserve a constant relation in most conditions, but that neutral fat varies widely. In hemorrhagic lipemia, the ratio *total fatty acids/lecithin* increased, whereas *lecithin/cholesterol* was not much changed. Bang (1918a) has placed on record his belief that the fat and cholesterol content of blood, and hence their relationship, are kept relatively constant.

The *cholesterol/cholesterol ester* ratio in blood plasma is one of the ratios of blood constituents which has been found to be quite constant. The percentage of cholesterol as ester in total cholesterol was found by Bloor and Knudson (1916) to be about 60 per cent. Boyd (1933a), in normal young women, found 70 per cent, and Sperry (1936a), in a recent extensive study (91 human adults), found values ranging from about 70 to 75, averaging 73 per cent with a standard deviation of 1.4 per cent. He concluded that the ratio is much more constant than has been generally supposed.

These are interesting suggestions and are supported by the limited amount of data on hand. Whether they will be found true as more values become available may safely be left until then. A review by Degkwitz (1931) of the data in the literature on the lecithin-cholesterol balance in tissue and in various reactions shows that, although a balanced relationship in physiological processes between these two substances is probable, sufficient exact data are not yet available to prove it. In the meantime, the conception serves as a useful basis for further work.

Normal Variations in Members of the Same Species

A summary of normal average values and constant ratios does not, however, complete the picture of normal conditions in blood. A plasma cholesterol value of half the average for the species in an otherwise normal individual may occasionally be found, and yet appears to have no significance as regards the well-being of the animal. Consequently, it is

important to know how much variation there may be in the various lipids among members of the same species and also in a single individual from time to time. Taking the data on experimental animals as more reliable than those on human beings, because animals are more controllable, Terroine's figures (1919) for normal dogs are given in Table 13. In this work samples were taken under standard conditions, and the Kumagawa-Suto procedure for total fatty acids and the Windaus digitonin method for cholesterol were used.

Table 13. Variations in Normal Dogs (Terroine, 1919).
(Mg. per 100 Cc. Moist Weight)

Page*	Number of Dogs		Av.	Range
327	16	Total lipids†	454	314–669
328–9	6	" "	429	353–495
348–9	7	" "	442	314–564
348–9	7	Total fatty acids	319	220–413
348–9	7	Cholesterol	122	90–152

* Page number in Terroine's monograph (1919).
† Total lipids = total fatty acids plus cholesterol.

Along with these results of Terroine may be presented those obtained by Bloor (1914, 1915, 1916a; Bloor, Gillette and James, 1927) making use of quite different methods, *i.e.*, the nephelometric method for total lipid and total fatty acids, and a colorimetric method for cholesterol. These methods both give somewhat higher values than the Kumagawa-Suto and Windaus digitonin methods, respectively; but regarding the first at least, the writer believes that they may be nearer the true values owing to the oxidative change and consequent loss of some of the fatty acids during the Kumagawa-Suto treatment. The experiments were made on normal dogs at least 14 hours after the last meal. Table 14 is a summary of average values obtained at four different times.

Table 14. Values on Blood Lipids of Dogs.
(Mg. per 100 Cc.)

Reference	Number of Dogs	Total Fatty Acid Av.	Total Fatty Acid Range	Phospholipid Av.	Phospholipid Range	Cholesterol Av.	Cholesterol Range	Total Lipids Av.	Total Lipids Range
				Whole Blood					
Bloor, 1914	9*							590	510–660
Bloor, 1915	5†	550	430–680	340	310–370	170	130–240	720	560–920
Bloor, 1916a	7*	590	520–680	350	320–400	230	180–300	820	700–980
				Plasma					
Bloor, 1916a	7*	620	540–760	350	280–500	220	150–370	840	690–1130
Bloor, Gillette and James, 1927	6‡	370	300–500	400	250–610	100	80–120	470	380–620

* Mixed diet.
† Ten experiments.
‡ Modification of the nephelometric method.

Lattes (1911), using the Kumagawa-Suto procedure, found that the average value for total lipids (total fatty acids plus cholesterol) in the

venous blood of 13 dogs was 382 mg. per 100 cc. with extreme variations from 303 to 428.

Mayer and Schaeffer (1913) found values for the serum of 4 dogs from 350 to 430 mg. of total fatty acids per 100 grams, moist weight. Cholesterol in the same animals ranged from 89 to 116 mg. per 100 grams moist weight.

In a series of six samples of normal dog plasma treated by direct saponification and extraction after acidification, comparable with the Kumagawa-Suto procedure, values of from 430 to 590 mg. of total lipid per 100 cc. of plasma were obtained by Bloor (1923). In a number of normal dogs used later for anemia studies, a series of fifteen complete lipid analyses by complete extraction with hot alcohol and saponification with extraction in petroleum ether using large amounts of plasma (100 cc. to 1700 cc.) gave values varying from 215 to 442, averaging 342 mg. per 100 cc. of plasma.

In conclusion, it is obvious that it is not possible to present satisfactory standard values for the lipids of dog plasma. Total lipid values average about 400 mg. per cent, but values from 300 to 600 mg. per 100 cc. may still be normal, and similar statements may be made for the individual constituents. Therefore, unless the normal range for a given individual is known, no values found in pathological conditions should be considered abnormal unless they are outside the figures for the species as given above.

Normal Variations in the Same Individual

Total lipid in normal animal blood may vary within wide limits in different species and in different animals of the same species. On the other hand, the plasma lipids of the same animal will vary much less from time to time than in different members of the same species or in different species. In Terroine's monograph (1919, p. 328), data are given on six dogs over periods ranging from four days to six months, in which the greatest variation from the average value for total lipid for the same animal was 169 mg. per 100 grams, and the extreme variation in values for one animal was 329 to 429 mg. per 100 grams or 30 per cent of the lowest value.

Also in a series of four animals (1919, p. 333), of which the blood samples had been taken over a period of several months, Terroine found the values for total lipids given in Table 15.

Bloor (1914) obtained similar values on differential animals at intervals of a week or more (Table 16).

In later work in this laboratory (Bloor, 1933), five dogs were kept on a constant fat-poor diet for over two years, the food intake being adjusted so that the weight remained practically constant. The averages

Table 15. Variations in Blood Lipids in Dogs (Terroine, 1919).
(Dry Weight in Mg. per 100 Gm.)
(Moist Weight in Mg. per 100 Cc.)*

	Minimum		Maximum		Percentage Variation	
Animal	Dry Weight	Moist Weight	Dry Weight	Moist Weight	From Lowest Value	Cholesterol/ Fatty Acid
I	1540	308	2263	452	46	6 4
II	1619	324	2126	425	31	0 0
III	1609	322	2072	412	14	0.0
IV	1705	341	2710	542	58	13.0

* Approximate moist weight values were obtained by dividing dry weight by 5. According to Terroine (1914a) the per cent of water in blood of normal animals is constant.

Table 16. Variation in Total Lipid Content of Blood (Bloor, 1914).
(Mg. per 100 Cc.)

Dog No.	Fat Content of the Blood at Various Times	Average	Percentage Variation from the Average
10	580; 610	590	2.0
13	670; 650	660	1.6
14	600; 600	600	0.0
15	600; 670	640	6.0
16	520; 530; 550; 610	550	10 0
17	510; 500; 600; 520; 580; 470; 490; 580	530	12.0
18	600; 640; 650; 600	620	3.3
19	550; 540; 540; 620; 600	570	8.0
20	500; 480; 560	510	10.0
Average for all dogs		590	
Greatest variation of each dog from his average			12 0
Average variation of each dog from his average			6.0

of seventy samples of plasma of two of the animals, distributed over all the months of the year except July and August, are given in Table 17. The methods used were the Liebermann-Burchard colorimetric for cholesterol and the oxidative for total lipid and phospholipid. In both animals, there was a pronounced drop in all lipid values during December and January and a steady rise during February to April. This is probably a seasonal variation not related to the diet, which was constant.

Table 17. Plasma Lipids of Dogs on Fat-Poor Diet Over a Two-Year Period (Bloor, 1933).
(Mg. per 100 Cc.)

	Phospholipid		Cholesterol		Total Lipid	
Dog	Average	Range	Average	Range	Average	Range
141	234 ± 27	198–300	133 ± 14	110–155	472 ± 40	390–572
199	169 ± 22	137–208	80 ± 14	60–109	337 ± 41	256–412

Human Blood

Normal values

The same factors which complicate the situation with regard to the interpretation of published figures on lipids in the blood of experimental animals are also present in the case of human blood, *i.e.*, different methods of analysis, uncontrolled conditions as regards immediate and remote influence of food, and individual variation. Under the circumstances, the

sets of normal values given by various workers, determined on samples from a limited series of normal human beings, agree fairly well. Some of the earlier values are given in Table 18.

Table 18. Normal Values for the Lipids of Human Blood.
(Mg. per 100 Cc.)

Author	Sex	No.	Total Fatty Acid Av.	Total Fatty Acid Range	Phospholipid Av.	Phospholipid Range	Cholesterol Av.	Cholesterol Range
			Plasma					
Bloor, 1916b	Men	14	380	300–430	220*	200–260	220	190–310
	Women	7	400	350–470	190*	170–220	240	210–260
Feigl, 1918c	Men	300			210*	120–320		
	Women	50			210*	140–295		
Oser and Karr, 1925		18			260*	190–350	190	110–260
Boyd, 1933a	Women	8	353	245–457	196	170–236	162	112–195
Man and Gildea, 1937	Men	4	320†	246–375	226*	174–250	203	151–237
	Women	6	345†	299–387	258*	241–277	226	171–249
			Corpuscles					
Bloor, 1916b	Men	14	360	280–450	400*	350–440	190	170–230
	Women	7	290	270–340	440*	390–480	210	190–240
			Whole Blood					
Bloor, 1916b	Men	14	360	290–410	300*	290–330	210	190–250
	Women	7	360	320–420	290*	280–310	230	210–240
Oser and Karr, 1925		18			340	280–410	150	110–190

* Obtained by multiplying phosphorus values by 25.
† Calculated as oleic acid.

Various segments of the blood picture have been reported on by separate workers, and they fit together fairly well. Wilson and Hansen (1936) give values as follows for seventeen normal sera: total lipid, 657 mg. per cent; unsaponifiable, 260 mg. per cent with iodine number of 63.4 (iodine number of cholesterol, 65.7); average molecular weight of fatty acids 291, with an iodine number of 108; 81 per cent of the phospholipid is readily saponifiable.

Brun (1936) reported that cholesterol in red blood cells of both men and women varied from 125 to 150 mg. per cent and was all in the free state. For white blood corpuscles in women, Boyd (1933b) reported the following mean values and ranges: total lipid, 1710 ± 734; fat 536 ± 536; phospholipid, 802 ± 255; total cholesterol, 300 ± 60; free, 194 ± 110; bound, 110 ± 97 mg. per 100 grams.

Greenwald (1915) found lipid phosphorus in four normal humans from 6.7 to 10.7 mg. per cent (167 to 260 mg. per cent of phospholipid). He found that, although the values varied greatly between individuals, they were quite constant in the same individual from time to time. Byrom and Kay (1927) reported 300 mg. per cent of lecithin in whole blood as a normal value. Thannhauser and Setz (1936) found 100-180 mg. per cent of sphingomyelin in sera containing 200-350 mg. per cent of total phospholipid. In the red cell stromata, the ratio of sphingomyelin to lecithin and

cephalin is about the same, *i.e.*, between 1.1 and 1.2 as in serum, but with wide variations.

Muller and Talbot (1931) investigated the effect of altitude on the blood lipids, but found no significant differences in cholesterol, lipid phosphorus, or total fatty acids in the blood of four healthy men at sea level and at an altitude of 10,000 feet for several days.

By a modification of the oxidative micromethods developed in this laboratory, Boyd (1933a) obtained the values in Table 19 for the complete analysis of the lipids of human plasma. The agreement with values obtained by the older methods is quite good, and these values probably represent as well as any a modern picture of the lipids of normal human blood plasma. It will be noted that the value for total cholesterol is considerably below the average value which the author had reported previously. No explanation can be suggested except the general and indefinite one of dietary differences. It has not been found that low cholesterol values mean anything unusual, but values above 250 mg. per cent in plasma generally are important.

Table 19. Plasma Lipids of Normal Young Women (Boyd, 1933a).
(Mg. Per Cent)

Total lipid	589
Neutral fat	154
Total fatty acid (I No. = 88.5)	353
Phospholipid fatty acids (I No. = 124)	130
Cholesterol ester fatty acids	77
Neutral fat fatty acids	146
Total cholesterol	162
Combined cholesterol	115
Free cholesterol	47
Phospholipid	196

Diurnal variations

It might naturally be assumed that the lipid values of the blood in any individual would vary during the day with relation to meals or fatigue; in fact, several workers have attempted to determine the amount of such variation. McClure and Huntsinger (1928) found only insignificant differences at hourly intervals in the blood cholesterol of normal young adult humans, but McEachern and Gilmour (1932) found large variations in the blood cholesterol of twenty-eight normal humans over five-hour periods. Bruger and Somach (1932), in a study of nine patients, examined the blood cholesterol at two-hour intervals for twenty-four hours and found that the whole blood cholesterol varied by a standard deviation of ± 8 per cent. The standard deviation for the morning hours was only ± 3.5 per cent. These were on samples taken while the patients were being fed normally. In a fasting group of nine subjects studied every

hour for four hours, the standard deviation was ± 3.9 per cent. Ingestion of food thus had little effect. They were unable to explain the diurnal variations found by McEachern and Gilmour, but stressed the fact that the results by the Liebermann-Burchard reaction are greatly influenced by temperature.

Boyd (1935a) took samples of blood three to four hours apart during the whole twenty-four hours, and found that the concentration of plasma lipids is not markedly affected by time of day, intake of ordinary meals, or sleep. Variations from person to person were found to be two or three times as great as the average variation per person per day. Man and Gildea (1937) followed the blood lipids in four males and six females over long periods of time and found extreme variations in cholesterol up to 31 per cent, lipid phosphorus 23 per cent, and fatty acids up to 37 per cent. The changes were not related to blood concentration since they were independent of protein changes; nor were they related to body weight, to the menstrual cycle, or to the season.

Anticoagulants

When mineral salt anticoagulants are used, there is always a danger that there may be dehydration of the corpuscles with consequent dilution of the plasma and corresponding low values. Gardner, Gainsborough and Murray (1938) found that potassium oxalate leads to a small shrinkage of the corpuscles, with values for cholesterol about 4 per cent too low (see similar results by Boyd, p. 127).

Lipids in the blood of various races

In Eskimos, Corcoran and Rabinowitch (1937) found the total cholesterol and phospholipid both somewhat lower and the *phospholipid/cholesterol* ratio somewhat higher than Boyd's normal figures. In natives of India (Bose and De, 1936), cholesterol was found to average 140 mg. per cent (120-160). In diabetics, values of 410 mg. per cent were found with little correlation with the degree of hyperglycemia. Low values for tropical races as compared with Europeans were reported by Radsma (1929).

Summary

The amount of data in the preceding pages is purposely extensive and is intended to bring out the fact that the "normal" lipid levels of blood plasma in man or lower animals are variable quantities, differing both with individual and species, and with nutritional factors, so that care must be taken in interpreting analytical results. For practical purposes, average values taken from analyses of a considerable number of individ-

uals of the same species under similar conditions are ordinarily used, and serve as a good basis for comparison; but the fact should not be overlooked that occasional normal individuals have values differing widely from this average. The safe way to compare the effects of experimental conditions on the blood lipids is first to obtain values from each individual under standard conditions.

Changes in the Postabsorptive Level Produced by Food

Effect of Fat Absorption on the Blood Lipids

The "normal" level of the blood lipids may be disturbed by a number of procedures, of which the most common is the absorption of fat from the intestine. Fat is delivered to the blood mainly by way of the chyle as a suspension of fine droplets of what is largely pure fat, no significant amounts of phospholipid (Eckstein, 1925) or of cholesterol being added to it during the absorption (unless there is cholesterol in the food). It is mixed with the circulating blood and remains in suspension there for a considerable time, constituting the blood dust or hemakonia of Neumann (1907), and Neisser and Braeuning (1907) and the chylomicrons of Gage and Fish (1924).

A study of the nature of these droplets, which are about 1 micron or less in diameter, has been made by Ludlum, Taft and Nugent (1929). They suggest that the chylomicrons may be stabilized by protein films, since the first zone of aggregation of the particles occurs at a pH between 4.7 and 5.3, which are approximately the isoelectric points of albumin and globulin, respectively, and coalescence takes place when the acid is sufficiently strong to destroy the film and precipitate the protein.

Although it is generally assumed as obvious that the increment of increased fat in the blood is the same fat that has been fed, it is known that some changes may take place in it during the process of fat absorption. Wilson and Hanner (1934) observed in normal children fed either cream (iodine number of fat 30-40) or cod liver oil (iodine number 165) that the increment of blood fat in the case of cream had an iodine number of 39-60 and in the case of cod liver oil, 118-135, indicating that the fat had undergone some change in the passage into the blood. As noted later, changes of blood lipids in response to absorption of fat take place, not only in the fat of the blood, but also, both in extent and nature, in the phospholipids and probably also in the cholesterol esters.

Effect on fat

The time of greatest blood-fat values after fat absorption has been found by different observers to vary from the second to the sixth hours

and apparently depends on the kind and amount of fat fed. Bang (1918c) found considerable differences in the nature of the response because of this factor. Thus, lard did not produce hyperlipemia in dogs; butter generally did, and olive oil always did. He also found great individual differences in dogs in their reaction to ingested fat. Most observers place the peak at the fifth to sixth hour after a relatively heavy meal of a well-absorbed fat, such as olive oil. In Figure 2 are given some typical absorption curves

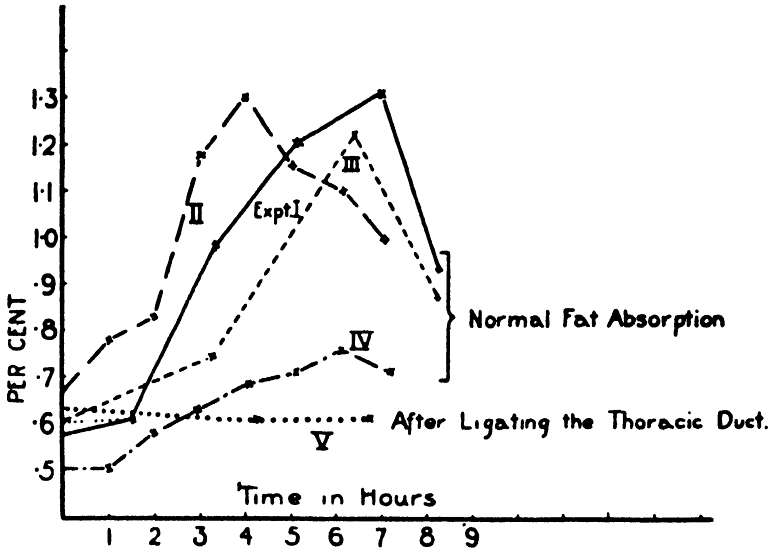


FIG. 2. Changes in the fat content of the blood during normal fat absorption.

showing increase of total lipid in the blood of dogs after olive oil (Bloor, 1914).

Man and Gildea (1932) have recently made a study of fat absorption in man after a fat meal containing 3.54 gm. of butter fat per kilo body weight. Some of their results (recalculated as mg. per 100 cc.) are as follows:

	Phospholipid fatty acids	Fat fatty acids
Before	210	213
2 Hours after	252	315
4 " "	294	315
6 " "	290	298

Effect on cholesterol

In a series of experiments published in 1914, Terroine (1914c) found that cholesterol increased during the absorption of fat in dogs and that the increases were parallel to those of fatty acids when the amount of fat

absorbed was moderate, the relation *cholesterol/fatty acid* being preserved constant. When the absorption of fat was large, the increase of cholesterol did not keep pace with it. Reicher (1911) found, in three experiments on dogs, great increases in lecithin (82 per cent) and cholesterol (65 per cent) during fat absorption, with an average fat increase of 53 per cent. Widal, Weill and Laudat (1912) found an increase in cholesterol esters but not in total cholesterol as the result of feeding olive oil. Hiller, Linder, Lundsgaard and Van Slyke (1924) found irregular increases of cholesterol in the blood of humans during fat absorption and no constant relation between fat and cholesterol. Bang (1918b) and Blix (1926), using Bang's methods, found no change in blood cholesterol in dogs during the absorption of large amounts of fat. Bloor (1915) reported a series of experiments in which the changes in cholesterol were not much, if any, beyond the limit of error of measurement in any of the experiments. However, in a series (Bloor, Gillette and James, 1927) undertaken as part of a comparative study of the reaction to a given fat-feeding of dogs before and after being made severely diabetic by removal of most of the pancreas, it was found, in seven experiments on six normal dogs, that cholesterol increased in six, and that in three out of the seven, the ratio *cholesterol/fatty acid* was fairly constant throughout the absorption. When the same animals were diabetic with already high cholesterol, the blood cholesterol generally increased along with the increase of absorbed fat, but almost always more slowly than the fat so that the cholesterol to fatty acid ratio decreased. Milbradt (1930) reported that feeding of triolein caused an increase in blood cholesterol which he believed to be the result of a washing-out process, since the amount in the adrenals diminished. Few of these workers paid any attention to the cholesterol content of the fat fed, but the increases are mostly too great to be accounted for by food cholesterol, especially since Kooy and Rosenthal (1933) have found that feeding cholesterol to rats for a short time caused no change in its constant level at 92 mg. per cent, feeding for eight days or more being required to produce a marked hypercholesterolemia. Blotner (1935) studied the blood cholesterol in a fat tolerance test using 500 cc. of 20 per cent cream and found that in normal or thin persons there was no rise of cholesterol in five hours, while in obese persons and those suffering from diabetes insipidus, there was a rise. Insulin caused a rise in cholesterol, but pituitary extract prevented lipemia. Turner and Steiner (1939) found the blood cholesterol in man remarkably independent of food intake during the day. A high cholesterol breakfast produced no effect and a high-fat diet only a doubtful rise. A low-fat diet had no effect. From the variable nature of the results reported, the occurrence of a characteristic increase in blood cholesterol after fat feeding is doubtful.

To explain the increase in cholesterol, when present, it has been claimed that there is a synthesis of cholesterol from fat, a claim for which, as Terroine has pointed out, there is no evidence. On the other hand, there are no known stores of cholesterol in the organism sufficient to cover these changes. Even the adrenals, which contain the largest percentage amount of all the tissues, do not contain enough to cover the observed increases in the blood unless it be assumed that they can also synthesize cholesterol (Chauffard, Laroche and Grigaut, 1920). The part played by the bile in supplying cholesterol to the blood has generally been overlooked, and as Bürger (1928) has shown, the cholesterol of the bile, if reabsorbed, as it probably is during fat absorption, would be sufficient to explain the increases in the blood found after feeding fat.

Effect on phospholipid

Less information is available regarding the effect of absorbed fat on the blood phospholipid. Reicher (1911) found a marked increase (32 per cent) of blood lecithin as the result of fat absorption, a greater percentage increase than he found for the fat itself. Most workers since then (Bang, 1918b, 1919; Hueck and Wacker, 1919; Zucker, 1920; Knudson, 1921; Bodansky, 1931; Man and Gildea, 1932) have noted an increase of lipid phosphorus in the blood during fat absorption. Hueck and Wacker (1919) reported that prolonged feeding of cholesterol resulted in increase of both lecithin and fat. Bloor (1915; Bloor, Gillette and James, 1927) found increases in lecithin in both plasma and corpuscles, and found the largest increases to be in the fat content of the corpuscles, which fitted in with the earlier finding of Munk and Friedenthal (1901) that the fat of the corpuscles increased during fat absorption. Knudson (1921) and Bodansky (1931) obtained similar results and also noted increases in cholesterol esters in the corpuscles. Boyd and Tweddell (1935) noted that there were variations in ester cholesterol and neutral fat in the red cells during the day, which led them to the belief that the red cells may assist in fat transport. Vahlquist (1931) was unable to confirm the increase of phospholipid in the corpuscles during fat absorption. More recent experiments in this laboratory were also negative in this respect. There is, however, no reason to question the positive results obtained earlier; and evidence supporting them is supplied by Artom and Peretti (1932), who, after feeding iodized fat or giving it intravenously, found always a greater increase both of iodized fat and iodized phospholipid in the corpuscles than in the plasma. The amounts, however, were not large. In summary, then, the evidence is positive with regard to the increase of phospholipid in the plasma during fat absorption, but an increase does not always take place in the corpuscles. In the light of the newer knowledge regarding phosphorylation

of the fat during its passage through the intestinal epithelium and later in the liver (see Fat Absorption) the presence of this extra phospholipid in the blood is to be expected and is to be regarded as fatty acid in transport.

Fat ingestion and the blood lipids in humans

As compared with the published results on dogs, the experimental blood response of normal human beings to the ingestion of fat is relatively small, which may probably be referred to the fact that the dogs were given a much larger dose of fat than humans would tolerate. Neumann (1907) and Neisser and Braeuning (1907) reported an increase in blood fat as determined by a count of the suspended fat particles (hemakonia), and these findings have been corroborated by recent extensive studies of the same kind by Gage and Fish (1924), McArthur (1930), and Hotta (1930). Using chemical methods, Cowie and Hoag (1921) found increases in the blood of children after feeding cream. Bang (1918b), Blix (1926), and Hiller, Linder, Lundsgaard and Van Slyke (1924) found only slight increases in normal humans after fat feedings of about 1 gram per kilo body weight. The latter group of workers found, however, in nephritics with high blood lipids, that the same fat feeding brought about a marked increase in the fat and lecithin of the blood but no change in cholesterol.

Page, Pasternak and Burt (1930) examined the other lipids, cholesterol and phospholipid, after a feeding of fat. They found that cholesterol showed the greatest increase at about the third hour. Phospholipids increased but irregularly. The iodine number of the blood fatty acids was unchanged by the absorption. The absolute changes were relatively small, which perhaps explains the lack of difference in iodine number. Rony and Levy (1929) studied the absorption of fat in obese individuals (90 lb. or more overweight) and found a considerable difference in behavior. Some did not react at all, some gave responses greater than normal individuals, and others behaved like normals. The total fatty acid content of blood was only slightly higher in these obese individuals than in normals. They found a close relation between alimentary lipemia and sugar tolerance. The greater the sugar tolerance, the less the effect of absorbed fat on the level of the blood fatty acid. Hyperglycemia, caused by ingestion of 100 grams of glucose, was found to be accompanied by variations in blood cholesterol by Mosenthal (1932), who thought that the fluctuations might be accounted for as compensatory osmotic phenomena. A positive picture of alimentary lipemia in humans was obtained by Man and Gildea (1932) using large doses of fat (3.54 grams per kilo). They obtained rises in plasma of 34 per cent of total fatty acids and 18 per cent phospholipid. Even 0.5 to 1.0 gram of fat per kilo, together with

an otherwise balanced meal, produced an average rise in total fatty acid of 21 per cent, with slight increases of phospholipid.

Factors Modifying the Effect of Fat Absorption

The increase of blood lipids during fat absorption varies considerably in different animals and to a less extent in the same animal from time to time, a finding which is to be expected from the normal variation in animals and also from the fact that the amount of fat in the blood, even at the peak of absorption, is a relatively small fraction of the total amount absorbed. Previous lipid levels, the rate of inflow and outflow, and the type of food fed with the fat are all interrelated factors which modify the response of the blood during fat absorption.

Removal mechanism

The amount of fat in the blood is the result of the balance between inflow and outflow, and would be expected to vary both with the rate of absorption and the rate of removal. Individuals with a blood lipid level already high, indicating an impeded outflow, generally tend to show an abnormally great increase in blood lipids in fat absorption, as was found in nephritic humans (Hiller, Linder, Lundsgaard and Van Slyke, 1924) and was also shown in extreme fashion in experimentally diabetic dogs (Bloor, Gillette and James, 1927). As an example of the latter work, an animal which normally showed a maximum increase in total fatty acids of from 320 to 378 mg. (or 58 mg. per 100 cc. of plasma) after being fed 100 cc. of olive oil, showed when very diabetic a change from a value of 905 to one of 5660 (or 4655 mg. per 100 cc.), a response nearly one hundred times as great. The response of blood cholesterol and lecithin was also greater in these animals but not nearly as marked as that of the fat. Thus, cholesterol increased in the animal, when normal, from 81 to 112 mg. (or 31 mg. per 100 cc.), and when diabetic, from 264 to 469 mg. (or 205 mg. per 100 cc.)—seven times as much—whereas the lecithin, which when normal had increased from 366 to 472 mg. (or 116 mg. per 100 cc.), showed in the severely diabetic animal a change of from 336 to 506 mg. (or 170 mg. per 100 cc.). (It may be noted that the lecithin level in this animal had not been raised as the result of his diabetes.) These accumulations of lipid in the blood have been explained as the result of an insensitive removal mechanism, and it has been observed in human diabetes that the feeding of a fat at a higher level sometimes results in a lowering of the blood lipid level, as though the mechanism were sluggish and required a greater than normal stimulus to set it working.

A mechanism for the control of the level of fat in the blood and the possibility of different metabolic treatment of different fats is suggested

by the work of Leites and his associates (1933, 1934). The effect of feeding a fat on the blood fat and on the blood ketone bodies was found to depend not so much on the amount of fat fed as on the initial level of the blood lipids and ketone bodies. If these were high, the feeding of a fat (butter) tended to lower the levels; but if the initial levels were low, it tended to raise them. Furthermore, butter or linseed oil produced no lipemia in dogs, though hemp or olive oil did. Butter produced a lipemia and ketonemia in man. On this basis, fats were classified as physiologically reactive or physiologically inactive. Such a regulation might explain results such as those of Chaikoff, McGavack and Kaplan (1934), who found no typical or uniform response in man from feeding olive oil up to 1.4 grams per kilo, either on the fat or cholesterol of the blood.

The fat on removal from the blood goes to the storage depots, with probably a short stay in the liver. To what extent the liver acts merely as a temporary reservoir, lowering the peak of the flood of fat, or to what extent it changes part of it (by phosphorylation) before releasing it, is not known. Fatty livers are common in untreated diabetes as is also a high lipid level in the blood. It is possible that the high blood level is the result of the filling of the liver with fat which thus blocks its further utilization. It is significant and suggestive that choline, a constituent of lecithin, prevents and, when present, removes the excess fattiness of the liver, as though the formation of lecithin with choline as the limiting factor were an essential step in fat metabolism in the liver.

Beumer (1923a,b, 1927, 1928) found no relation between blood lipase (esterase) and nutritional disturbance; in fact, he expressed a doubt as to whether this so-called lipase had anything to do with fat metabolism.

Shope (1928a) found cholesterol esterases (enzymes which hydrolyze cholesterol esters) in blood serum and in many tissues. In blood after death, the esterase may cause the complete disappearance of the esters. Sperry (1935), on the contrary, found that the cholesterol esterase of blood plasma acted to increase the percentage of cholesterol as ester.

Lipid destruction in the lungs

It has been claimed by many workers, mostly of the French school, that blood lipids are destroyed in their passage through the lungs. Roger and Binet (1922) analyzed dog blood before and after its passage through the lungs and found a considerable loss of lipid during the passage. They suggest that the lungs play a role in the catabolism of the fats analogous to that of the liver in the catabolism of proteins and carbohydrates. Rémond, Colombies and Bernardbeig (1924) found that the blood of the right side of the heart was always richer in cholesterol than that of the left. It seemed, therefore, that cholesterol is either fixed or destroyed in

the lungs. Abelous and Soula (1921) found that the blood of the right heart contained more cholesterol than that of the left. On the other hand, Markowitz and Mann (1930), after examining the blood going to, and coming from the lungs, could find no changes significant of any part played by the lung in fat metabolism. Shillito, Bidwell and Turner (1936) could find no differences in blood cholesterol in the carotid artery, venae cavae, and portal vein, and therefore no destruction in the lungs. They give a good review of the literature on this debated point. Bugnard (1929) gives the following figures: The average excess of cholesterol in the venous blood of the dog as compared with the arterial is 13 mg. per cent. The average excess of cholesterol in the corpuscles of the arterial blood over that of the corpuscles in the venous blood is 40 mg. per cent. He concludes that during the passage of blood through the lungs, there is a shift of cholesterol from plasma to corpuscles, which he thinks explains the supposed destruction. Bugnard (1930) explains the mechanism as follows: Cholesterol is a regulating factor in the physico-chemical equilibrium of the blood correlated with changes in pH, and its distribution between plasma and corpuscles depends on pH. As the blood gives up its carbon dioxide in the lungs, cholesterol passes from plasma to corpuscles and the reverse takes place in the tissues.

Nutritional state and diet

The nutritional state of the animal at the time of feeding and the effect of feeding other foodstuffs along with the fat are of importance in the effect of fed fat on the blood lipids. Rony and Ching (1930) found that fat-feeding experiments on dogs 15 hours postabsorptive gave a marked variance in response, but that a preliminary 7- to 14-day fasting period rendered the responses the same in all. Ordinarily the amount of fat fed (2 cc. of olive oil per pound of body weight) gave a good alimentary lipemia, but the lipemia was much less if at the same time dextrose (1 gram per pound of body weight) was given either intravenously or parenterally. Insulin reduced the alimentary lipemia; enough insulin to lower the blood sugar to about 30 mg. per cent prevented alimentary lipemia, although it produced no effect on the fasting blood lipids. Rony and Ching conclude that the process of removal of carbohydrate from the blood favors the removal of fat. Results similar in kind were reported several years ago by Bang (1918c), who found that the feeding of bread along with fat inhibited the lipemia, and that a liver rich in glycogen prevented the accumulation of fat in the blood. In rabbits and guinea pigs, Versé (1917) found that there was ordinarily no lipemia during fat absorption, but Schoenheimer (1929) found that he could produce a lipemia in rabbits if sodium desoxycholate was fed along with the fat, indicating that the

ordinarily slow absorption was due to a lack of bile salts in the intestine of these animals.

The interrelations of fat and other food during absorption was investigated by McClure and Huntsinger (1928), who found that fat ingestion influenced protein and carbohydrate metabolism, as indicated by diminution of non-protein nitrogen, urea and sugar, as was found also by Leites and his associates (1933, 1934). Dextrose ingestion caused a lowering of amino acid nitrogen. Cholesterol and total fatty acids increased in the blood after every type of food, the greatest increase being after oleic acid and the least after a fat-free meal. Feeding oleic acid caused an increase of blood phospholipid.

An interesting side-light is thrown on the behavior of the blood lipids by the work of Gabbe (1921) in carrying out the so-called stimulative therapy. Fluctuations in blood lipid content were found to occur regularly after injection of collargol, caseosan, hypertonic sodium chloride, glucose, sucrose, argochrome, and horse serum. Both phospholipids and cholesterol are apparently involved. Small doses which cause insignificant temperature reactions may produce an increase in blood lipids lasting several hours. Larger doses cause an early decrease and then an increase above the original concentration.

Intravenous Injections

Many experiments on intravenous injection of various lipids are reported in the literature, and one may be given as typical of the kind of experiment and the results. Nomura (1929) found that injected fat disappeared quickly from the circulation except a small amount which persisted up to 24 hours. Neutral fat, cholesterol, and lecithin were removed at the same rate. There was no effect on blood sugar, blood pressure, or respiration, and ordinarily no effect on the respiratory quotient or heat production. Only after a 7- to 9-day fast was there any increase in the amount of fat oxidized or decrease in the amount of protein.

Pasternak and Page (1932) gave massive injections of cephalin intravenously to rabbits and found that the lipid content of the plasma was increased and remained high for a long time. The phospholipid excess disappeared sooner than that of the other lipids. Half an hour after a large cephalin injection, the phosphatide content of the liver was greatly increased and the phospholipid was later replaced by fat. Small injections of cephalin over a long time did not change the blood phospholipid, although the total quantity of cephalin injected may have been great.

Holt, Tidwell and Scott (1935) experimented with injections of fat into humans. They used fat very finely emulsified with lecithin to a par-

ticle size less than 2 microns. Since the proportion of lecithin was high (about one-third), some of the effects obtained were probably due to it. They found that the injection produced a sudden and great increase of blood lipids with a fall to about half within one hour and then a slow fall to the normal level or below in about four hours.

Allen and Wishart (1923) injected the lower fatty acids—butyric, isovaleric, β-hydroxybutyric, and diacetic—free and as sodium salts, and found that the injection was followed by dyspnoea, drunkenness, coma, and death. The symptoms were specific and not due to acidity, because the salts were also fed, and because the symptoms were not the same as those of acid poisoning. Moreover, acetone produced the same symptoms, including a lowering of plasma bicarbonate.

Cashin and Moravek (1927) found that the injection of colloidal cholesterol (3 per cent solution with 0.3 per cent lecithin) had a profound physiological effect. The blood cholesterol dropped 20 to 60 per cent and the animal became insensitive to pain. Respiration rate increased and heart rate fell. A similar fall in blood cholesterol was found by Klein and Levinson (1933) as the result of injections of India ink, indicating that the drop in cholesterol, and possibly other effects, may be a general colloidal phenomenon.

Effect of Diet on the Blood Lipids

The effect of a single feeding of fat is to produce definite but transitory changes in the lipid picture of the blood. It is desirable to know the effect of the continuous feeding of fat at a relatively high level. Would it produce a permanent elevation of the blood lipids, and might dietary factors explain the wide variance in plasma lipid levels in different animals which have been recorded? White and Hunt (1930), in their study of diabetic children, found that the blood cholesterol of children on an adequate mixed diet was lower than when there was a large amount of fat in the diet. Overnutrition increased blood cholesterol, and extreme variations from the normal standard body weight were accompanied by excess of cholesterol in the blood. Mjassnikow (1926) found that a large feeding of cholesterol (8 eggs) daily produced no change in the blood cholesterol of healthy subjects, a finding which was in agreement with the results of Gardner and for which confirmation was obtained by Hunt (1929). In rats, however, Kooy and Rosenthal (1933) found that feeding cholesterol for eight days resulted in a marked cholesterolemia, although a shorter period had no effect.

Results on dogs have been reported by Ling (1931), who found that the blood lipid decreased on a fat-free diet, the change being due to lipids other than lecithin or cholesterol. During starvation, there was a still

further drop in lipids, and in this case, the phospholipids dropped markedly and the cholesterol less. With olive oil, the values rose for all the lipids, except free cholesterol, which fell, because of the increase in the cholesterol ester fraction. A ration low in fat caused a lowering of the total fatty acids and cholesterol in the blood of cows, with a correspondingly lowered milk yield (Maynard and McCay, 1929). Hansen, Wilson and Williams (1936), in work on dogs, confirm previous workers in showing that the level of serum lipid depends on the intensity of fat metabolism. All the principal fractions of the total lipid take part in the fluctuations to about the same extent, indicating that all are concerned in the transport and utilization of the fat. Coconut oil produces a higher lipid level than linseed oil. The iodine number of the fat being transported in the blood reflected closely that of the fat fed, and leads to the belief that neutral fat in the blood is the main transport agent, and that the phospholipid is probably concerned with the ultimate combustion of the fat.

Further experiments to provide definite evidence regarding the effect of diet on blood lipids have been carried out in this laboratory on two widely different species of animal: the dog and rabbit (Bloor, 1932). Dogs are accustomed racially to a great deal of fat in their diet, rabbits to a diet mainly vegetable, in which there is very little fat. Dogs digest fat easily and readily develop an alimentary lipemia, whereas it is difficult to produce an alimentary lipemia in rabbits (Versé, 1917). For these experiments, the animals were kept on: (a) a diet very poor in fat, and (b) a diet rich in fat (fat equal to one-third to one-half the caloric intake) for several periods of one to two months each, blood samples being taken every three or four days, sixteen hours after the last food was taken. It was found that on the high-fat diet, the levels of all the lipids were higher in both species than on the low-fat diet. In the dogs the differences were not great, but in the rabbits they were very marked; for example, total lipids in the rabbits were two to three times as great on the high-fat diet as on the low. Some very low values were obtained for rabbit plasma, for example, cholesterol values of 15 and 18 mg. per cent. The percentage of total cholesterol present as ester was always higher in the dogs on the high-fat diet than on the low, but in the rabbits the percentage was not changed. As an example of how low the cholesterol ester can be in a typically herbivorous animal may be quoted the figures of Gardner (1924) on hippopotamus blood. The total cholesterol was 38 mg. per 100 cc. of blood, of which less than 1 mg. per cent was present as ester.

The effect of diet on the blood lipids in human beings has not been experimentally studied. In general, their blood lipid picture compares quite closely with that of the dog, and probably for the same reason: that

racially they are adjusted to a considerable proportion of fat in their diet. Whether on a low-fat, vegetable diet their blood lipid values would be different remains to be shown. However, the war diet in Germany was found by Rosenthal and Patrzek (1919) to bring about a reduction of blood cholesterol. Rosenthal (1919) found also reductions in cholesterol of the corpuscles from the normal of 0.4-0.66 to 0.124-0.327, which he regards as due to chronic undernutrition.

In calves, it has been found that there was a correlation between the lipid content of the blood and the mean milk fat production of their mothers (Schoorl, 1935).

A prolonged low-protein diet caused no alteration in the blood lipids of dogs, even when the serum albumin fell below 1.5 per cent (Page, Farr and Weech, 1937). On the other hand, an exclusive meat and fat diet, over a year, such as that used by Stefansson and Andersson, was found by Tolstoi (1929) to produce a lipemia and hypercholesterolemia which returned to normal when the diet was discontinued.

It is evident that dietary factors must be considered in the case of some of the abnormal values occasionally found in otherwise normal human beings.

Variations in Blood Lipids in Normal Individuals

The Reproductive Cycle and the Blood Lipids

Much work has been done on the effects of the several stages in the reproductive cycle on the blood lipid level in the lower animals in the attempt to find some correlation between lipid changes and fertility and lactation. In the case of humans, the effort has been made to determine normal levels and variations and to connect abnormal differences with bodily changes. This work has been loosely classified under appropriate headings; but it is obvious, because of the disconnected nature of the data, that a complete picture of any of these topics is not yet possible.

Menstruation and ovulation

Okey and Boyden (1927) found a fall in blood cholesterol during or within a few days of the menstrual period, followed by a blood cholesterol level higher than the normal average for the individuals concerned, changes which were not consistently paralleled by the rise and fall in fatty acids and phospholipid. These results have been confirmed by Kaufmann and Mühlbock (1929), who reported a fall of blood cholesterol at the menstrual period of about 40 per cent. In the pregravid stage of the cycle there was a rise of 50 per cent in disturbed ovarian function. In lues and after the menopause, this rhythm in the blood lipids was absent. They found no

change in blood phospholipid during the cycle, which is in agreement with most of the results in the literature.

The blood plasma of female fowls has been found to contain more alcohol-soluble substances and more phosphorus than males, and that of actively laying fowls much more than others (Lawrence and Riddle, 1916). The relative values of phosphorus were as follows: males 100, non-laying females 115, and laying females 205; ratio of alcohol-soluble substances: males 100, non-laying females 116, and laying females 181. The poorest-laying hen had blood poorest in these constituents. These investigators also cite literature to show similar differences in blood of various animals during active ovulation. These values are definitely to be regarded as an expression of function. Riddle had previously found differences in the lipids of male-producing and female-producing eggs (see Lawrence and Riddle, 1916). Riddle and Burns (1927) found that during ovulation, the ether-soluble substance of ring dove blood increased 35 per cent, the phosphorus content increasing 50 per cent. Normal males had shown blood fat of 1.77 per cent, normal females 2.02 per cent in the resting stage. Parhon and Parhon (1923a,b; 1924) found higher blood cholesterol in hens preceding the laying period. Lorenz, Entenman and Chaikoff (1938) found that just before egg laying started in the young hen, there was an enormous increase in blood fat up to 2781 mg. per cent, with an increase of phospholipid to 967 mg. per cent, but with cholesterol changes much less. In laying hens, the total lipid also showed enormous variations; in one bird 131 days after laying began, the total lipid reached a value of 4129 mg. per cent with phospholipid 1485. Cholesterol, however, exceeded the 200 mark only twice. The values in the non-laying females and males were: total lipid about 460, total cholesterol 118 with fluctuating values for cholesterol ester, and phospholipid about 290 mg. per cent. In later work, Lorenz, Chaikoff and Entenman (1938) found that estrin raised the blood lipids in both the immature female and the male. With 3000 rat units, the values in the female doubled in 12 hours. In the male after 2000 units, the rise was 1000 mg. per cent. All lipids shared the rise, but it was most marked in the neutral fat.

Gestation

Oshima (1907) found that the fat in the blood of guinea pigs increased during the later weeks of gestation and not before, and also that the increase occurred whether the animal was fed or not. Kaufmann and Erdmann (1932) found no difference in blood cholesterol between pregnant and non-pregnant rats. Coope and Mottram (1914) found that the fat in the liver of rabbits was considerably increased in the later stages of preg-

nancy and early in lactation. The pituitary as a factor in this transfer of fat has been studied (Coope and Chamberlain, 1925), and it was found that the injection of pituitrin into rabbits caused an increase in the fat of the liver. Chamberlain (1929) found that the adrenals of female rabbits contained more cholesterol than those of males.

Increased blood lipids have been practically always found in pregnancy in humans. Thus Herrmann and Neumann (1912) found that the total lipid, neutral fat, and cholesterol esters (especially cholesterol palmitate) were higher than normal in the blood of pregnant women, while the lecithin was about the same. Their figures are given in Table 20.

Table 20. Blood Lipids in Pregnancy (Herrmann and Neumann, 1912). (Mg. per 100 Grams)

	Free Cholesterol	Ester Cholesterol	Lecithin	Total Lipid
Normal non-pregnant	86.4	57.6	238	590
Near end of pregnancy	83 5	97 1	225	780
New-born	78.1	14.1	205	437

Boyd (1934) reported in his series that the increase is almost entirely in the plasma. It begins during the first trimester of the pregnancy with increase in phospholipids and cholesterol in the second trimester. At term, the neutral fat is most increased (over 100 per cent) with no change in composition (iodine number). Boyd (1937) found also that in incomplete abortion in women, there was an increase in plasma lipid values compared with those in the corresponding period of normal gestation. The increase was mostly in the cholesterol ester, free cholesterol, and phospholipid fractions. Knauer (1928b) also found that the phospholipid increased toward the end of pregnancy.

Data on the time relations of the lipid changes are furnished by Tyler and Underhill (1925), who found that the total ether extract of whole blood in pregnant women is higher than in non-pregnant as early as the third month, and at term averages 50 per cent higher. Cholesterol, cholesterol esters, and lecithin increase to term when each is one-third higher than at three months. The ratio of cholesterol to cholesterol ester remains fairly constant. In view of the work of Lorenz and associates (see p. 148) on the effect of estrin in greatly increasing the blood lipids even in males, the increase noted in gestation may possibly be an estrin effect.

The extent of combination of cholesterol with plasma protein has been found to increase greatly toward the end of pregnancy (Eufinger, 1928). If a certain proportion of free cholesterol is necessary for the proper functioning of blood, the increase of cholesterol in pregnancy may be explained as due to the fact that part of the cholesterol present is tied up with protein.

The puerperium and lactation

Slemons and Stander (1923) found that at the end of labor in seven cases delivered without anesthesia, the total cholesterol of the mother's blood varied between 200 and 300 mg., while in fetal blood the total cholesterol was 130 to 180 mg. per cent, much less than in maternal blood. Rosenbloom's (1935) figures also agree, being 223 mg. per cent for maternal blood and 120 mg. per cent for fetal blood. Slemons and Stander found, moreover, that when the delivery was conducted without the use of an anesthetic, there were no cholesterol esters in fetal blood, or if present, the quantity was so minute it could not be estimated. Shope (1928c) found the same in the blood of calves: a complete absence of cholesterol esters at birth and a rapid increase during the early life of the animal. The work of Boyd and Wilson (1935), however, goes to show that there is a passage of lipids across the placenta in humans. Phospholipids, free cholesterol, and ester cholesterol pass into the fetus, while neutral fat may pass either way.

During the first two weeks of the puerperium, the lipids in the maternal blood remain high. Slemons and Stander (1923) offer the suggestion that the increase in blood lipids represents a preliminary step in the preparation for lactation. They find that the mother's blood contains much more lipid than the fetal blood. The difference which exists between the two organisms in this respect varies from case to case, but in no instance were results obtained which indicated an equilibrium or a free exchange between the two circulations. The human placenta is generally regarded as impermeable to fat and other lipids (see later report by Boyd), although the placentas of other animals seem to be permeable to some fats. Fetal fat, including cholesterol, then, must be synthesized, probably from glucose which is freely supplied by the mother. On the other hand, the non-protein nitrogen, amino acids, urea, creatinin, and glucose values in maternal and fetal blood indicated that the concentration of these substances in both circulations was approximately the same and that there was, therefore, a free exchange of these small-molecular, diffusible substances.

György (1926), Plass and Tompkins (1923), and Hellmuth (1926) report very much lower values for lipids in the blood of the new-born than in the blood of the mother. These values change quickly after the birth of the child, the values in the mother's blood falling while those of the infant rise. In human milk of the first week there is twenty times as much cholesterol as in the fifth week, after which the values stay constant. These findings all point to the increase of lipid in the mother's blood as a preparation for lactation, particularly for the early days of lactation, during which time the infant makes up the deficiencies in its own blood

lipids from those supplied by the early milk. Similar provision for the offspring in the blood of the mother is noted by Parhon, who found in hens that there was an increase in blood cholesterol before the laying period, and that the values fell during the laying period (Parhon and Parhon, 1923a,b, 1924). Also note work by Riddle and associates (p. 148) and Chaikoff and associates (p. 148).

In lactation, it has been noted (Schaible, 1932) that the values for total fatty acids, phospholipid and neutral fat were much higher in lactating than in non-lactating animals, while the nature of the fatty acids was much the same. The content of fatty acids in the phospholipid was too low for the typical phospholipid. The iodine number of the fatty acids in cholesterol esters was too low for linoleic acid alone and, therefore, a mixture of esters was probably present. As is usually the case, a larger proportion of unsaturated acids was in combination with cholesterol than in the phospholipids. Leroy and his associates (1931) found a high coefficient of correlation between the total fatty acids in blood and the butter fat content of milk, and suggested the fatty acid level of the blood in young animals as an indication of the future milking capacity of cattle. Schoorl (1935) found that there was a correlation between the lipid content of the blood of calves and the mean milk-fat production of their mothers.

The increase in blood lipids during pregnancy occurs in most species but is not a constant phenomenon; but lactation in mammals and egg production in birds is always associated with increased blood lipids and is to be expected because of the greater amount of fat being secreted.

Age

In babies at the end of one to one and one-half months, the lipid values are up to 50 to 70 per cent of the adult values, and after three to four months they do not change in the remainder of the year. In the following years, the values rise slowly. György (1926) found that phospholipid rises more rapidly than cholesterol during the first two weeks after birth, then cholesterol increases more rapidly. Sperry's (1936b) series from birth up to 25 days showed an average cholesterol value of 135 ± 25 (71-190) as compared with adults of 209 ± 50 (130-150). The *cholesterol ester/cholesterol* ratio is lower than in adults and seems to increase with age. The most pronounced increase in total cholesterol was in the first four days. Cowie and Hoag (1921) found, in children of three to eleven years, that the total lipid value was about the same as that of adults. Age after maturity does not appear to have any certain effect on the blood lipids. Thus while Parhon and Parhon (1923a) found blood cholesterol increasing with age, Blix (1926) found no difference.

For young children of various ages, the figures in Table 21 are supplied by Gordon and Cohn (1928).

Table 21. Change of Blood Lipids with Age (Gordon and Cohn, 1928).
(Mg. per 100 Cc.)

	Cholesterol	Phospholipid*
At birth (cord blood)	89	102.5
First week	87	105
5–12th month	136	152.5
5–6th year	169	177.5

* Phospholipid values = lipoid P × 25.

Ward (1931) found that in boys of six to thirteen years of age the cholesterol increases with age. In girls, greater variations were found. The amount was increased in chronic rheumatism and chronic parenchymatous nephritis. Kaiser and Gray (1934) corroborated the results of others in finding that the lipid content of the blood of children 5-16 years old was lower than that of adults.

Molitch and Poliakoff (1936) determined the blood cholesterol in 505 boys aged 8 to 18 years. Their results show that in 284 normal subjects, the serum cholesterol (total) varied from 81-204 mg. per 100 cc. with values of 100-160 mg. in 85 per cent of the subjects, the average for the entire group being 130.7 mg. and the median value 127.5 mg. No difference was found between white and negro boys. These workers found that the level was influenced by the fat in the diet. High values were found in hypothyroidism.

Green and Macaskill (1928) found that the lipid phosphorus in calves was the same as, or higher than, that of the mother at birth and increased in the first few weeks after birth. The blood globulin in calves behaves similarly, being low at birth and increasing rapidly to nearly adult level. The fact that there has been demonstrated a combination between the lipids and the euglobulin of blood may be significant in this connection. These variations are of the same order as those which Mayer and Schaeffer (1914) found in young rats, and indicate that young animals begin life with a low blood lipid level which is greatly increased during the early period of life and then more slowly, reaching the adult level, however, quite early.

Work

Murlin and Riche (1916) reported that the total lipid of the blood of dogs at first fell below (first half hour) and then increased above normal during work, returning to normal after an hour's rest. In human beings, various workers have found that the blood lipids increased with exercise. However, Robinson, Brain and Kay (1927) found that exercise reduced

blood cholesterol: in whole blood from 190 to 168 mg. per cent, in plasma from 172 to 149 mg. per cent, *i.e.*, a reduction in both corpuscles and plasma. The values returned promptly to normal after exercise. Patterson (1927) found that blood cholesterol increased up to 40 per cent as the result of exercise, and that this rise could be prevented by feeding sugar. Stewart, Gaddie and Dunlop (1931) reported that the total fatty acid of blood rose after sufficiently intense exercise and that with it there was a fall in the respiratory quotient. Unfortunately, the method for total fatty acids used by these workers has since been shown to be defective (Long and Venning, 1932), which raises some question as to the correctness of both their work and that of Patterson. It would naturally be expected that the demand for fuel produced by hard work would bring about an increased mobilization of fat, raising the blood fat level. Gage and Fish (1924) showed by definite increases in the fasting chylomicron count (probably neutral fat) that vigorous exercise increases the fat of the blood. It is however possible that the removal of fat by the working muscle, unless balanced by an equal or greater inflow from the stores would result in a lowered blood lipid, especially in the early stages of the exercise.

Effect of Abnormal Conditions on the Blood Lipids

Since the discovery of the blood circulation, and particularly since the development of methods for the analysis of very small quantities of blood, an obvious point of attack for almost any problem has been a study of the blood constituents. This has been especially true of the many pathological conditions because of the diagnostic possibilities, and every clinician of a scientific turn of mind who happens on an interesting case promptly has the blood analyzed. The resulting literature is very confusing, but there is much of value if it were made available. In the following section, an attempt has been made to collect the most important of the work on the lipid constituents of the blood. The whole field of abnormal conditions has been divided into convenient groups. First will be found all the conditions associated with nutritional abnormalities: undernutrition, overnutrition, and various imbalances; next is a group of infections; and finally a group of organic diseases, including their laboratory counterparts. These divisions are often arbitrary but will perhaps serve the purpose of making this part of the subject more comprehensible.

Nutritional Abnormalities

Possible nutritional abnormalities range from complete lack of food and water to an excess of either or both, and include partial deprivations or surpluses of all or any of the nutritional elements. Beginning with a

discussion of simple fasting, there will be considered the effect on the blood lipids of conditions such as anemia and cancer which result in general malnutrition, then the effect of the lack of certain substances as found in vitamin and mineral deficiencies and diabetes, the effect of surpluses as in obesity, and finally a discussion of water balance and epilepsy as it affects the blood lipids.

Fasting

The animal organism in fasting depends for maintenance mainly on its stores of fat. The available stores of carbohydrate and protein are small and are quickly exhausted, so that after the first two or three days in the larger warm-blooded organisms and in a shorter time in the smaller ones, the individuals are living on their fat stores and on their body protein. These facts mean a considerable movement of fat from the stores to the points where it is used, and since the blood is the transporting medium, it might be expected to show changes in its fat or other lipid content. Actually, such changes are found to be irregular, sometimes appearing and sometimes not, a peculiarity which, as has been shown by Leathes, may be due to the fact that the amount of fat transported is relatively so small, when considered in relation to the volume of the blood and the time, that it might not be demonstrated by the methods used to measure it. On the other hand, increases of fat in the blood do occur at times and may be demonstrated by as simple a procedure as counting the fat particles (chylomicrons) with the aid of a dark field microscope (Gage and Fish, 1924). The explanation offered by several observers (Bang, 1918b; Bloor, 1914; Terroine, 1914c) who have noted this irregularity is that the increase, if any, will depend on the condition of the fat stores. If the stored fat is easily movable, the stimulus of starvation will cause its discharge in larger amounts than can be used at once, so that it accumulates in the blood. As the amount of easily movable fat diminishes, the effect should disappear.

Comparing an animal of the same species with another, which is a doubtful procedure in view of the differences in blood lipids in individuals (see Tables 13 to 17), Schulz (1897) found in fasting a 50 per cent increase in the blood lipids of rabbits and 20 per cent in that of pigeons. In a long-continued fast on the same animal, Daddi (1898) found an increase in ether-extractable material in the first week of the fast, followed by values below the normal level, lasting until the death of the animal. Terroine (1914c), in a series of dogs fasted to the point of death, examined the water content of the blood and the blood lipids (cholesterol and total fatty acids) and found that the blood often showed variations in its

content of water, cholesterol and fatty acid during the fast. The extent of the variations was different in different animals. Cholesterol tended to become lower, especially at the beginning of the fast. Ellis and Gardner (1912) found in the rabbit a higher value for cholesterol in fasted animals than in normally fed ones. Bloor (1914) found that the fasting of dogs (from five to seven days) may or may not produce an increase in the blood fat (total fatty acids plus cholesterol) depending apparently, as above noted, on the nutritional conditions of the animal, since one animal which had not shown any change in a previous fast exhibited a marked rise in blood fat after he had been overfed with fatty food for a week before the fast. Lattes (1911) and Freudenberg (1912) found sometimes an increase and sometimes no change in the blood lipids after short fasts in dogs. Underhill and Baumann (1916) found a decrease in blood fat at the beginning, followed by a rise to normal values as the fast proceeded. No relation between blood fat and blood sugar could be noted.

In undernourished patients (Man and Gildea, 1936), the cholesterol level varied with the state of nutrition, being below normal in most emaciated patients, and rising with improvement of nutrition. Total fatty acids were below normal in most of the emaciated patients. Phospholipid phosphorus varied with cholesterol. Hypocholesterolemia was usually accompanied by low protein and albumin. Shope (1928b) found that fasting caused a hypercholesterolemia which feeding brought back to normal. He found also that a straight fat diet for 48 hours did not increase blood cholesterol, which would indicate that cholesterol is not concerned in fat metabolism. Sure, Kik and Church (1933) found in rats fasted to death that the blood cholesterol remained essentially normal, phospholipid fell (200 to 150 mg. per cent), and total fatty acids fell (340 to 200 mg. per cent). Gregg (personal communication), in a study of a human being who fasted 57 days and died of bronchopneumonia, found that all blood lipids fell, both in plasma and corpuscles. Total fatty acids in whole blood fell from 440 to 300 mg. per cent, in corpuscles from 160 to 60. Cholesterol fell in both with a rather sharp rise near the end. Phospholipid fell in whole blood from 240 to 120, in corpuscles from 180 to about 60 mg. per cent. The liver lipids at the end were notably low: phospholipid 1.86 per cent, cholesterol 0.15 per cent, as compared with 2.8 and 0.28 in normals. Great variations in response to starvation are apparent, as might be expected from the different nutritional states of the animals and the differences in their mode of response to the stimulus of starvation. In general, there is a tendency to increase in the blood lipids in the early part of the fast, followed by a decrease. In human beings, the tendency is toward a decrease throughout.

Anesthesia and narcosis

The earlier work on the effect of narcotics and anesthetics on the lipids of the blood contains some important ideas and may therefore be reviewed in some detail. Bibra and Harless are quoted by Reicher (1908) as stating that the ether-soluble material of the brain was decreased and that of the liver increased after repeated ether anesthesia, and they came to the conclusion that material was dissolved out of the brain and transported by the blood to the liver, a conception which was plausible enough to hold the field until displaced by the Meyer-Overton theory of narcosis. The increase in power of the blood to dissolve lipids, due to dissolved narcotics such as ether, is still believed to be an important factor in the production of high blood lipid values. Reicher found that the blood fat might be increased up to 300 per cent by various narcotics and that the result was due about equally to fat, cholesterol, and lecithin, a reaction which he regarded as a protective mechanism, the lipids in the blood dissolving the anesthetic and so protecting the cells. This suggestion was put to experimental test by Nerking (1909), who found that injection of lecithin did have a marked effect in increasing the amount of narcotic necessary and in shortening the recovery period, a result which Kramer (1913) with more careful technique was unable to confirm. Lattes (1911) was unable to confirm Reicher as regards chloroform anesthesia, finding no increase in blood fat, even in a 1.5-hour period. Bloor (1914), using 3-hour periods of anesthesia with ether and chloroform, found with ether a pronounced rise in the blood lipid in all cases but found none with chloroform in dogs on a diet low in fat. In the one animal which had been on a high-fat diet for a week, there was a marked rise in the blood fat under chloroform. Alcohol and morphine produced no observable effect on the blood lipids during the narcosis; but in the case of morphine (and also of chloroform) there occurred next day or the day following a pronounced rise, which may perhaps be referred to the damage to the tissues, especially the liver, which has been found to happen after chloroform by Howland and Richards (1909) and by Whipple and his associates (1919).

Ducceschi (1920) found that alcohol administered for some days to a dog produced regularly a marked hypercholesterolemia and a distinct increase of fatty acids in the blood. The phosphatide content was less markedly and constantly increased. There was a consistent increase in the total fat of the liver which might amount to 300 per cent of normal; the cholesterol and dry residue were increased to a less extent. The adrenals showed a constant decrease in cholesterol (average 40 per cent), and a slight increase of total fat and dry residue. No characteristic or

constant changes could be observed in the lipid content of kidneys and testes, although two young dogs showed marked cholesterol increase in the kidneys. These changes were favored by inanition. Human beings who indulge in alcohol in excess show an increase of blood cholesterol as compared with those who use it moderately or abstain. Repeated etherizations of 60 to 90 minutes per day for several days produce a notable increase of serum cholesterol which may last for several days after. Chloroform similarly used causes the death of the animal within eleven days with greatly damaged liver and a rapid fall of blood cholesterol.

Cattoretti (1915) found no immediate increase in blood fat during the anesthesia, but a maximum increase about one hour afterward which varied in extent and duration with the length of the anesthesia. Two factors were recognized as affecting the change: the behavior of the individual, and the time of feeding. In one animal which died under the anesthetic, the increase was rapid during the narcosis.

Feigl (1918e) found that acute alcoholic poisoning led to lipemia. The change was mainly in the plasma and developed in three steps: (1) hyperlecithinemia (3 to 8 hours); (2) hypercholesterolemia—an inflow of free cholesterol; and (3) high fat (15 to 18 hours). Liquidation was relatively slow. The first two steps as stages in pure intoxications were also seen in liver atrophy. The erythrocytes took up some lecithin at first but afterward lost it. In chronic alcoholism, only 20 per cent showed visible lipemia and 66 per cent a moderate increase in blood lipids. The total lipid was always above 1 per cent. Twenty-eight per cent showed no lipemia. In more marked cases, both lecithin and cholesterol were increased.

Mahler (1926) found in ether anesthesia a rise in blood cholesterol parallel to the rise of glucose and the duration of anesthesia. Insulin prevented the increase; this seems to connect insulin with the cholesterol level of the blood.

The somewhat discordant results reported above on the effect of anesthesia and narcosis on the blood lipids may be referred to a number of factors which may be summed up as follows:

(1) The effect of the narcotic on the solubility of lipids in the blood. Ether and alcohol dissolve in blood plasma in concentrations great enough to increase its solvent power for lipids; chloroform is much less soluble and would have less effect than ether; morphine would not be expected to change the solvent powers of the blood for the lipids.

(2) The effect of the narcotic on the fat stores. It might either cause or prevent a discharge of fat into the blood.

(3) Interference with the gas exchange in blood and tissues by actual prevention of the entry of oxygen into the blood in respiration (partial

asphyxia) or by prevention of the transfer of oxygen and carbon dioxide into and from the tissues.

(4) Injury to tissues by the anesthetic, which is well recognized after chloroform and which may happen as the result of derangement of the processes of cells produced by the presence of the narcotic. The tissue most noticeably and frequently affected is the liver. The damage done by an anesthetic would depend largely on its relative solubility in blood and tissues. Taking ether and chloroform as examples, both are readily soluble in the lipids of tissues; but ether is much more soluble in blood than is chloroform, so that it would be more easily removed from the tissues after the anesthesia was over and its damage to the tissue would be correspondingly less.

Anemia

All types of anemia will be discussed here, since the final result of any type is the same as regards the blood lipids.

Experimental anemia in animals. Much study has been devoted to changes in the blood lipids in the anemia produced by bleeding. Boggs and Morris (1909), experimenting with rabbits, found that when anemia was produced by bleeding 25 cc. per day for eight to sixteen days until the blood corpuscle count was reduced to below two million, the blood serum became milky and contained 2.45 per cent of fat. The fat of the blood could not be centrifuged out or extracted with ether until the calcium was removed by ammonium oxalate. It could, however, be collected by precipitation of the protein with alcohol. The fat had the following properties: specific gravity 0.93, iodine number 105-134, free acid 0.85 per cent, lecithin 10 per cent, and cholesterol none.

Horiuchi (1920b) repeated Boggs and Morris's experiments with rabbits, paying particular attention to the blood lipids, and followed the lipemia through its course. As a control measure he (1920a) studied the blood lipid changes in the blood of normal rabbits over considerable periods of time. Two series of anemia experiments were run: one on a high-fat diet and one on a low-fat diet. His results may be summed up as follows: Values for total lipids in the fat-fed anemic rabbits were found to be enormously greater than those of the same animal when normal, in one case reaching a value twenty-five times as great as the normal. In anemic rabbits on a low-fat diet, the increases were not so great as on the high-fat diet but were still relatively enormous, in one case reaching to about eight times the normal value. The differences in behavior on the two diets indicated that the fat of the food took part in the lipemia. The peak of the lipemia was reached in about three days after the onset, and the milkiness had practically disappeared in another

three days, although high values with clear plasma persisted for a long time. The increases in fat were accompanied by similar large increases in lecithin and cholesterol but these did not increase in proportion to the fat. The corpuscle values remained nearly normal, indicating that the corpuscles took no part in the disturbance. Chamberlain and Corlett (1932) produced anemia in rabbits by bleeding and by phenylhydrazine poisoning; in both the blood cholesterol first fell, then rose to figures several times the normal, and this rise was part of a general lipemia.

Schmitz and Koch (1930) found that in hemorrhagic anemia in rabbits the greatest increase in the phospholipid was in the lecithin. Cephalin increased, but to a less extent, and the final values were very near those found by Waksman (1918) to be the optimum for blood coagulation. Milbradt (1930) found that the lipemia in this condition was due to fat mobilized from the tissues, of which the skin yielded most, then the spleen, brain, kidney, lungs, and adrenals. The increase of cholesterol found in bone marrow was due to the change of yellow (fatty) to red tissue. Fishberg's (1929) suggestion as regards nephrosis that the extra lipids act vicariously for the lost protein in maintaining the colloidal osmotic pressure of the blood may find application in these hemorrhagic lipemias. However, Schwarz and Lichtenberg (1937) submit evidence that the lipemia is not due to changes in the blood protein, since in their experiments these were kept constant by reinjection of the serum, but rather to the anoxemia and the resultant blocking of fat combustion.

A single massive bleeding in rabbits (Boyd and Stevenson, 1937) caused a drop in phospholipid and cholesterol esters in the first three hours which was restored in twelve hours. The drop in these lipids was greater than the drop in hemoglobin. Cholesterol fell with the hemoglobin and was back to normal level in twelve hours. Neutral fat rose above the normal level and remained high.

A study of the differences in dog blood when normal and after being made anemic by repeated bleedings was reported by Bloor (1923, 1924, 1925). It was found that the outstanding feature of the lipids of anemic as compared with normal plasma was the increased percentage and degree of unsaturation of the unsaturated fatty acids in combination in the various compounds in the anemic plasma, a fact noted earlier by Csonka (1918). It should be emphasized that the increase in unsaturated acids was the result and not the cause of the anemia. The greatest percentage differences were noted in the lecithin fraction, which averaged about 30 per cent higher in amount and 25 per cent higher in iodine number in the anemic animals than in the normal ones. The iodine number of the fatty acids of the neutral fat (glycerol esters) was also higher, while that of the fatty acids of the cholesterol ester fractions was unchanged.

No milkiness of the plasma was noted in the dogs, although the Tyndall effect was markedly increased. Unsaponifiable matter averaged about 8 per cent higher in the anemic animals than in the controls.

Terroine (1920) bled three dogs four times at intervals of two or three days, removing one-fourth to one-half of the total blood each time. The animals were not fed for 18 hours before each bleeding and the diet was low in fat. He found, in spite of the severity of the treatment, that cholesterol and total fatty acid percentages of serum at the end were very little different from the beginning values. The repair mechanism seemed indeed to work better with the lipids of the serum than with the other solids. However, serum cholesterol did increase as the result of the bleeding, a change which was more marked when the values were calculated to dry weight. In the corpuscles, he found that the increase of both total fatty acids and cholesterol was very marked, which indicated that the new cells were much richer in these lipids than the old ones. There was thus an efficient regulation in the serum, since in one case an amount of blood equal to the whole original volume had been withdrawn.

Very high values were reported for hemorrhagic anemia in the guinea pig by Feigl (1921): fat ten times, lecithin four times, and cholesterol over seven times the normal values. Feigl also found high lipid values in humans as the result of hemorrhage. The lipemia in hemorrhagic animals, observed especially in herbivorous animals, is apparently the result of an outpouring of fat from the body depots secondary to the anemia. The excess of fat in the blood is accompanied, as in fat absorption, by increases in phospholipid and cholesterol in the plasma. The changes in cephalin percentage, as pointed out by Schmitz, are seemingly adapted to limiting further hemorrhage.

Dubin (1918) produced anemia in dogs by trypanosomes and found that the total lipid increased, while the lecithin and cholesterol diminished, which points also to a mobilization of fat from the depots.

Summary. In experimentally anemic animals there is a characteristic increase in the blood lipids, especially the fat, and an increase in the degree of unsaturation of the fatty acids. The explanation for this may well be the slowing of oxidation with consequent piling up of the more difficultly combustible substances (the lipids) in the blood. The extent of the accumulation would depend on the rate of discharge from the depots as well as the rate of removal from the blood. The rate of discharge would be proportional to the need of energy.

Human anemia. In a study of anemia of various origins in human beings, Bloor and MacPherson (1917) found that as long as the percentage of corpuscles was not below half its normal value, the amounts of blood lipids were normal. When the corpuscle percentages fell below that value,

abnormalities appeared, which, in the order of their frequency as well as of their extent, were:

(1) High neutral fat in plasma:
(2) Low cholesterol in plasma and occasionally in corpuscles;
(3) Low lecithin in plasma.

Corpuscle values remained essentially normal. The relation between free and bound cholesterol remained normal. Removal of the spleen resulted in an increase of total fatty acids in the corpuscles and of cholesterol in the plasma. MacAdam and Shiskin (1923) likewise found no diminution in blood cholesterol until the corpuscle values fell below 50 per cent of the normal level. There was no notable difference in the effect of pernicious and secondary anemia.

In anemia resulting from loss of blood in human beings, Feigl (1921) found very considerable increases in blood lipids: fat up to eighteen times the normal value, and lecithin and cholesterol up to about six times the normal value, the lecithin to cholesterol ratio remaining practically normal. Apparently human beings react to severe hemorrhages in the same way as rabbits and guinea pigs, while dogs are more resistant.

In children with anemia, according to Knauer (1928b), the lipids are low, especially cholesterol. In the growing organism, the blood-forming organs show the effect of dietary deficiencies sooner than other tissues.

The changes in blood cholesterol in pernicious anemia and the effect of remissions on the cholesterol level have been studied by Muller (1930). She found that accompanying the rise in reticulocytes during remission there was a sudden rise in blood cholesterol lasting for about 12 days, then a second rise to normal which was maintained as the reticulocyte count fell to normal. Lecithin phosphorus followed parallel to cholesterol, while the fatty acids remained at their high level throughout. The change in blood lipids is closely related to the extent of the remission, no matter in which way it is produced. Macy and associates (1937) report that in pernicious anemia there is an increase of neutral fat with a deficiency of phospholipid and cholesterol esters in the plasma, while in the corpuscles in relapse the cholesterol ester is high and the free cholesterol and phospholipid are deficient. After therapy the values in both return to normal levels. Von Szent-Györgyi and Tominaga (1924), in studies of the genesis of pernicious anemia, believe that they have shown a relationship of the condition to the amount of free, polyunsaturated fatty acids. They find in normal blood no fatty acids yielding bromine addition products insoluble in ether, whereas some are found in pernicious anemic blood. In the hemorrhagic anemic blood of dogs, Bloor also (1923, 1924, 1925) found

an unusually large proportion of unsaturated fatty acid which was, however, the result and not the cause of the anemia.

As far as any conclusion is possible at present, it seems that the changes in blood lipids are secondary to the anemia and not the cause of it. The increases of blood cholesterol accompanying the remissions in pernicious anemia which Muller found might then be the result of factors which produced the remission rather than that of the increased cholesterol acting to protect the red blood corpuscles and prevent their destruction, a role that has usually been assigned to cholesterol. Other suggestions to explain the increased blood lipids found in anemia are: (1) that they are the result of the diminished oxygen-carrying power of the blood which results in diminished energy production in the tissues and brings about a mobilization of fat to supply the deficiency and produces the emaciation often found in anemia; and (2) that the change in the lipid would compensate for the loss of the blood proteins in adjusting the colloid osmotic pressure of the blood (Fishberg, 1929).

Cancer

The blood lipids in cancer have been examined by several workers and the reports are conflicting. Mattick and Buchwald (1928, 1929) found the cholesterol higher in plasma than in whole blood in 85 per cent of cancer patients. In 80 per cent of normal patients, it was higher in whole blood. They later reported a tendency to hypercholesterolemia in cancer plasma with little change in corpuscles. Lecithin was slightly lower in the corpuscles than in plasma but not significantly so. Downes and Pack (1932) could find no constant relationship in this respect, nor was the cholesterol content of the blood increased in cancer.

Guthmann (1930), in 229 cases, found a relation between the course of the cancer and the level of the blood cholesterol, *i.e.*, that cholesterol decreased in early carcinoma and markedly decreased in late cases. De Voss (1932) found phospholipid, and ester and free cholesterol somewhat lower in cancerous than in cancer-free patients, except in the case of carcinoma of the liver, gall bladder and pancreas in which higher values, except of cholesterol esters, were noted.

Kaufmann and Erdmann (1932) could find no difference between normal and tumor-bearing rats as regards their blood cholesterol. The free cholesterol averaged about 68 mg. per cent and the esters 20 mg. per cent. In the pregnant rat the tumor grew more slowly. Similarly Dannenberg (1932) could find no significant difference in the blood of cancerous and normal rats. The values for both averaged about 75 mg. per cent for free cholesterol and 19 mg. per cent for ester cholesterol.

Burgheim (1927) found that x-ray radiation produced lowered blood

cholesterol in cancer cases, but that in benign affections there was no change.

In summary, it may be said that up to the present no definite relation between blood lipids and incidence or rate of growth of tumors has been demonstrated.

Vitamin deficiencies

Avitaminosis. Collazo and Bosch (1923) found that after five weeks on a vitamin-free diet, dogs showed a slight hypolipemia. This was followed by a stage of hyperlipemia with its maximum in eleven to thirteen weeks. Thereafter, the blood fat decreased markedly, almost reaching the normal value at the end of the sixteenth week, when death occurred. Similar results were obtained with guinea pigs. Feeding 16 grams of lard to dogs with avitaminosis was followed by an increase in blood fat which was later more prolonged, and less in extent, the more advanced the avitaminosis. Onohara (1925) reported that hyperlipemia was a frequent but not regular accompaniment of avitaminosis, and Asada (1923) concluded that in this condition there was a disturbance in the power of the cells to take up fat. Milbradt (1930) reported the following findings in avitaminosis: that cholesterol increased in muscle, liver, and skin; that phospholipid decreased in all parenchymatous organs and in brain, but remained constant in muscles; and that cholesterol esters increased in liver and skin.

Cramer (1920) described "lipid gland tissue" found in the white rat: (1) at the back of the shoulders between the scapulae, (2) in the thorax along the aorta, (3) in the abdomen between the kidneys and the abdominal aorta and surrounding the adrenal glands which lie imbedded in it. In rats, dead as a result of a vitamin-free diet, both fats and other lipids may be completely lacking in this tissue. It is suggested that these lipid glands form an important deposit of one or more of the vitamins.

Vitamin B deficiency. Iwatsuru (1924) found that when animals were deprived of vitamin B, the plasma lipids rose (in agreement with the findings of Ogata and Lawaczek in pigeon beriberi), cholesterol increased to three times the normal value, total fatty acids rose to three times the normal, and cholesterol esters increased along with the free cholesterol. The composition of the corpuscles remained normal. Schmitz and Hiraoka (1928) found high values for cholesterol in muscle and brain which they think may be residual, because of the loss of tissue substance. Life could be prolonged in these cases by lecithin injections, which also prevented diminution of red blood corpuscles and hypertrophy of the adrenals. The two organs which do not atrophy as the result of the lack of vitamin B, the adrenals and the hypophysis (McCarrison, 1919), are organs closely

related to lipid metabolism. According to Lecoq (1933), the assimilation of lipids requires the presence of vitamin B.

Scurvy. Ssokoloff (1924) found the blood cholesterol low in scurvy, coming back to normal on convalescence. According to Ohata (1932), scurvy in guinea pigs results in increased fatty acids and phospholipid in the blood with no change in cholesterol except in very advanced stages.

Vitamin A deficiency. Takahashi (1922) found that the nutritive value of cholesterol, lecithin, cephalin, and protagon, in the absence of vitamin A, depended upon the nature of the fatty acids. It was not a function of the melting point of the fats, but was closely related to their molecular weights. Among the glycerides of non-volatile fatty acids, the greater the molecular weight of the fat, the less its nutritive value, whereas in the glycerides of the volatile fatty acids, the reverse was the case. In general, the smaller the saponification number, the less satisfactory was the fat. Some lipids, like cholesterol, had an antigrowth effect.

Irradiation

Ultraviolet irradiation of children was found by Leopold, Bernhard and Tow (1932) to increase the blood fat in one-quarter of the cases. Inunction with wool fat followed by irradiation produced an increase in blood lipids in most of the low ones and in one-third of the normals. Phospholipids were unchanged. Kultjugin (1927) found that irradiation of rabbits increased their blood fat 500 per cent, while cholesterol was increased only 30 per cent.

Magnesium deficiency

Kruse, Orent and McCollum (1933) found that a deficient magnesium intake produced profound and fatal metabolic disturbance in the rat and dog. While the total lipids of the blood were unchanged, the total cholesterol increased about 65 per cent. The increase was mainly in the cholesterol esters, which increased from a normal 60 per cent of the total cholesterol to 85 to 90 per cent. Total fatty acids decreased while phospholipid remained constant.

Diabetes

Diabetes mellitus. In this disease, more consistently than in any other, the presence of excessive fat in the blood has been reported, but it has been discovered only comparatively recently that along with the fat there are present also abnormally large amounts of lecithin and cholesterol—that the hyperlipemia is also a hyperlipoidemia (Fischer, 1903; Klemperer and Umber, 1907). Extremely high values for blood lipids have been reported in diabetes. Thus Imrie (1915) found in one

sample of diabetic blood, neutral fat plus cholesterol 14.1 per cent, cholesterol 1.5 per cent, and no cholesterol esters or lecithin. Neisser and Derlin (1904) found 19.7 per cent of fat in venous blood of a diabetic, and in a report by Chase (1927), the value of 40 per cent determined by the Babcock method for milk, was given. Herbert (1935) gave values of 22 per cent for fat, 1.41 per cent for cholesterol of which one-third was free, and 0.95 per cent for phospholipid. Feigl (1918d) found 1 per cent as his high value for lecithin in diabetic lipemia (with 15 per cent total lipid), the average for normals being 0.22 per cent. Boyd (1928) found that the fat (total fatty acid) of the blood of diabetic children was generally higher than normal. During acidosis, the values reached 1 per cent, and in coma, 2 per cent. There was no constant relation to blood sugar, but increased sugar tolerance tended to stabilize the blood lipids.

During the treatment of the disease by the Allen method of reducing body weight to a level which could be taken care of by the decreased pancreatic hormone, and by the use of insulin with dietary regulation, the great abnormalities in blood lipids in human diabetes largely disappear. Thus, Gray (1924), as the result of the examination of the data on one thousand samples of diabetic blood, comes to the conclusion that "strikingly high blood fats are astonishingly infrequent"; but he finds that the blood fat was above normal in treated diabetes with a consistency equal to that of blood sugar, 78 per cent having blood fat above the level of high normals, while 72 per cent had blood sugar above the high normal for blood sugar. The higher the blood fat level, the shorter the expectancy of life, and conversely, since only those patients live long who have low blood fat, the longer the duration of the disease, the lower the blood fat.

Blix (1926) came to the similar conclusion that a high degree of hyperlipemia was a rare symptom, while moderate or slight hyperlipemia was a relatively frequent one. Hyperlipemia was found in all of six cases of coma but in extremely varying degrees. Hyperglycemia is, he believes, a more sensitive indicator of disturbed metabolism than hyperlipemia. His work, along with that of other investigators such as Blatherwick (1921), Marsh and Waller (1923), and Freyberg, Newburgh and Murrill (1936) shows decisively that high-fat diet has little relation to the hyperlipemia, at least after the first few days when presumably adaptation is taking place. A high-fat feeding might even cause a fall in blood lipids, as reported by Bloor, Gillette and James (1927) in one of their diabetic dogs. Blix considers the hyperlipemia to be entirely secondary to the disturbance in carbohydrate metabolism. In diabetic dogs (Bloor, Gillette and James, 1927) a notable hyperlipemia was generally not found until the blood sugar was at about 250 mg. per cent, which

recalls the conditions laid down by Allen for the production of hyperlipemia in dogs: that the animal must be severely diabetic with high blood sugar. The lipemia in diabetes is generally accompanied by a fatty liver which indicates that the mechanism for handling mobilized fat has broken down and the result is an accumulation of fat, primarily in the liver, followed by an accumulation in the blood (see discussion of fatty livers in Chapter IV).

The excess fat in the blood in diabetic hyperlipemia appears to be ordinary food or tissue fat, as shown by the iodine absorption value. Thus, Fischer (1903) found that the iodine number of the fat in diabetic hyperlipemia (total ether extract 18.4 per cent, cholesterol 0.5 per cent) was about 60. Imrie (1915) found in a diabetic blood with a total lipid content of 14 per cent and a cholesterol of 1.5 per cent that the fatty acids had an iodine value of 65, which is about that of the fat in the depots. The possibility that the fat particles in the blood in the lipemia of diabetes may be large enough to be the cause of embolism has been noted by Bantin (1926).

Experimental diabetes. Most of the experimental work on diabetes has been done on dogs, and the first worker to experiment extensively on blood lipids in diabetic dogs was Allen (1922). He found that diabetic dogs which digest high-fat diets regularly develop some degree of hyperlipemia, and in a minority this lipemia becomes extreme (15 per cent or more of blood fat). Apart from a sufficient supply of fat in the diet, the one indispensable prerequisite for diabetic lipemia in dogs was the presence of active, severe symptoms in the form of glycosuria and hyperglycemia. Diabetic lipemia evidently represents some secondary breakdown in fat metabolism not directly connected with the endocrine function of the pancreas and not due merely to excess of fat in metabolism or to loss of sugar from the body. Wide differences in individual susceptibility to the hyperlipemia are shown both by dogs and humans. One-sided fat diets may produce fatal disturbances without significant hyperlipemia in dogs not severely diabetic.

Bloor, Gillette and James (1927) carried out a series of long experiments on dogs, studying first their blood lipid level and their blood lipid reaction to a standard dose of fat while normal, then rendering them severely diabetic by removal of nine-tenths of their pancreas and dieting, and after the operation observing again the changes in blood lipid level and in reaction to a standard fat dose. The most characteristic feature was always the elevation of the postabsorptive level of the blood lipids in the diabetic animals, and the fat was the constituent most markedly affected. The cholesterol level was often not abnormally high over considerable periods of the diabetic life of the animal, which is in contrast

to the high levels often found in human diabetic plasma and suggests other factors than fat in the latter case. The lecithin level was little affected by the diabetic state. Great hyperlipemia did not appear to be characteristic of the diabetic state, since most of the animals were severely diabetic over considerable periods without showing hyperlipemia. On the other hand, all but one of the animals showed it at some stage, and the hyperlipemia was accompanied and followed by a period of marked illness which was often succeeded by death, a fact which suggests that the lipemia was connected with the illness rather than with the diabetic state itself.

As regards the blood lipid response to the standard fat doses, it was often not excessively great in the diabetic animals unless they were already hyperlipemic, in which case, the increase in blood fat after the dose and the time of subsidence of the increase were often much greater than in the animal when normal. The facts with regard to the behavior of the severely diabetic animals toward ingested fat seemed to indicate a sluggishness of the removal mechanism in the blood and tissues, rather than a breakdown in fat metabolism. The relation between high blood sugar and high blood fat has already been noted (p. 165).

Chaikoff and Kaplan (1934) determined that the blood lipids in depancreatized dogs were all low, especially the ester cholesterol, which in most of their animals had disappeared entirely after three weeks, although it persisted in the liver to the extent of 0.17 per cent (moist weight) or 63 per cent of the total cholesterol. The total lipid of the liver in these animals was 21 per cent of the moist weight, and 75 per cent of the cholesterol was esterified, showing an accumulation of ester cholesterol in the liver. They found also (1935) that feeding raw pancreas to these dogs caused a rise in blood lipids which was most striking in the case of the cholesterol esters. Deprivation of raw pancreas resulted in a pronounced drop in all the lipids. The effect on the blood lipids of pancreatic factors other than insulin thus seems probable and is in line with the effect of raw pancreas in curing and preventing fatty livers in dogs.

Insulinization. It is well known that insulin reduces the blood fat of lipemic diabetics, although whether the result is direct or secondary to the improvement of the general condition is not known. Rabinowitch and Mills (1925) reported a case of diabetes with great hyperlipemia in which treatment with 70 units of insulin reduced the plasma fat from an initial 18.6 per cent to 9.4 per cent and four hours later to 2.1 per cent, which led them to conclude that the insulin acted directly by increasing the permeability of the tissues for fat. Fonseca (1924) had reported similar results. Curtis, Sheldon and Eckstein (1933) found a close rela-

tion between insulin and the disappearance of lipemia in a severe diabetic with extreme lipemia (lipid = 14.2 per cent) and xanthomatosis. The lipemia was readily controlled by insulin. White (1926) found that insulin lowered blood fat in severe diabetes and that age and weight had no bearing on the response. He found that a high-fat diet tended to raise the blood lipids. Page, Pasternak and Burt (1931) gave insulin to rabbits to the point of convulsions and found that all the blood lipids were lowered, especially phospholipid. In one rabbit, no trace of phospholipid could be found in the blood. Feeding of cholesterol in oil (Lewin, 1935) raised the blood cholesterol in rabbits, and the lipid phosphorus to a less extent. Insulin aided in assimilation of the cholesterol.

It appears impossible at present to state definitely whether the fat metabolism is directly under the control of the insulin or indirectly so through the effect of insulin on carbohydrate metabolism, although the weight of evidence is in favor of the latter. One fact, applying also to the hyperlipemia of nephritis and possibly to that of anemia as well, is that hyperlipemic individuals, whether humans or lower animals, are generally very sick and that improvement in their clinical condition by whatever means (even by feeding fat) generally results in a reduction in the hyperlipemia.

Effect of various drugs

Phlorizin. Lattes (1911) found a considerable increase in blood fat after phlorizin poisoning. Terroine (1919) fasted dogs for two or three days, then injected phlorizin and took a blood sample some hours after the injection. He found in three out of four dogs that there was a rise in total fatty acids and cholesterol and that the rise was marked in two. The changes in cholesterol were independent of those of the total fatty acids as shown by the variations in the cholesterol to total fatty acids ratio. Allen (1922) found that in spite of the fact that the fattiness of the liver, kidney, and heart was fully as great in phlorizin diabetes as in real diabetes, no real lipemia was ever present. Due to the loss of carbohydrate in phlorizin diabetes and in pancreatic diabetes, the behavior of the blood lipids should probably be the same as in fasting. Himwich and associates (1932) found that the muscles of phlorizinized or depancreatized dogs removed fat from the blood. The liver removed fat from the blood of fasting and phlorizinized dogs but added it to the blood in depancreatized dogs. The effect of phlorizin on phosphorylation, for example in the liver, may be an important factor in the accumulation of blood and liver fat.

Phosphorus. It is well known that phosphorus often causes a mobilization of fat and its accumulation in the liver. Lattes (1911) found

higher values for blood fat in phosphorus-poisoned dogs than in normal ones. Mansfeld (1909) found that it increased in only one dog out of four. Apparently the effect of phosphorus on the blood lipids is variable and subject to the same influences as affect fat mobilized by other agents.

Hydrazine poisoning. Underhill and Baumann (1916) found that the blood fat comes to a maximum corresponding to the hypoglycemia and the maximum corresponds to the flow of fat to the liver when its glycogen content has been lowered.

Acids. Injection of acid to the point of shock was found by Van de Veld (1928) to cause an increase in blood lipids, whereas injection of sodium bicarbonate caused a lowering.

Snake root or rich weed (Eupatorium Verticaefolium). The weed commonly known as snake root has been found by Bulger, Smith and Steinmeyer (1928) to produce lipemia in cows eating it.

Tartrates. Rose (1924) found that tartrate caused great increases in blood cholesterol accompanied by diminished kidney function. The same effect was produced by glutaric acid salts; but malic, malonic, and succinic acids were without effect.

Deposits of metabolic excesses

Xanthomatosis. Xanthoma, a disease characterized by yellow nodules on the skin, notably of the eyelids, is a frequent complication of diabetes, and in these cases the blood lipids, particularly cholesterol, are generally above normal. Thus, Yamakawa and Kashiwabara (1922) found that in the cases studied the cholesterol and neutral fat content of the serum was three times the normal, and the phospholipid content was above normal. A large amount of free cholesterol was found in the tumor masses and approximately as much cholesterol ester, but only a trace of phosphatides. Rosen and Krasnow (1932) also found rather high values in the whole blood in xanthoma: cholesterol 232 mg. per cent; lecithin 286 mg. per cent.

A form of xanthoma has recently been described under the names of essential xanthomatosis and essential cholesterolemia. The outstanding characteristic of the disease is the formation of nodules, in various places in the body, which contain large amounts of cholesterol. The blood cholesterol is very high—four or five times normal—and the other lipids are also high, but not in proportion to the cholesterol. The cause of the condition is apparently a congenital inability to excrete cholesterol. In some cases, the high blood cholesterol will disappear when the individual is put on a vegetable diet, which is free of cholesterol. On the other hand, cases have been reported which did not respond (Sperry and Schick, 1936). Chanutin and Ludewig (1937) found the blood cholesterol esters and

neutral fats reduced and free cholesterol, phospholipids, and total lipids increased in xanthomatosis. Treatment with a fat-free diet caused a moderate reduction on the blood lipids but had no effect on the nodules.

Other lipid deposits. Other disorders of lipid metabolism, which perhaps may be due similarly to an inability to burn the particular compounds which are deposited, are called Gaucher's disease, in which the cerebrosides accumulate, and the Niemann-Pick disease, in which lecithin and sphingomyelin are deposited. These conditions will be described more fully in a later chapter.

Muscular dystrophy

In muscular dystrophy, which is characterized in some animals by calcium deposits in the muscles and is apparently caused by some nutritional imbalance, Morgulis and Spencer (1936) found that the lipid phosphorus and cholesterol content show marked increases in diseased rabbits. These changes reverse on recovery.

Fattening and obesity

Knauer (1928a) found that all the blood lipid values were low in the hog while fattening. The changes were mainly in the serum, corpuscle values changing only slightly. The sedimentation rate increased in forced fattening as long as the organism could take care of the fat; when a constant weight was attained the values remained stationary.

Arnoldi and Collazo (1924) found in obese persons low blood lipid values, which is in accord with Knauer's (1928a) finding in pigs that the the fatter they were, the lower the blood lipids. Knauer thinks that the low blood lipids may be the result of an unusually good mechanism for removal of fat from the blood. Rony and Levy (1929) found that obese persons varied greatly in their response to a meal of fat, but no more than normals, and that obesity *per se* produced no effect on the blood lipids. On the other hand, Nissen (1930) found that obese persons generally gave a greater response to a given dose of fat than normals. As regards cholesterol, Bruger and Poindexter (1934) found the content normal in uncomplicated obesity and not changed by reductions in weight. Overnutrition in diabetic children increased blood cholesterol, and extreme variations from the normal standard body weight were accompanied by excess cholesterol in the blood (White and Hunt, 1930).

Epilepsy and water balance

The relation of cholesterol to water balance and its effect in epilepsy and related conditions has received attention. Mayer and Schaeffer found in tissues that the higher the cholesterol content, the greater the

ability of the tissue to take up water. If this rule held for blood, a low blood cholesterol would mean a decreased ability of the blood to retain water and hence an increased tendency for water to exude into the tissues. A tendency for the epileptic to retain water in his tissues during the active phase of the disease has been noted by McQuarrie (1929), who also found that restricting the water intake had a beneficial effect in controlling the seizures. [Fetterman and Kumin (1933) and Wilson and Limberger (1933) found that restriction of fluids was not effective.] Haustein (1929) found that the cholesterol content of the blood of eight children with disturbed water metabolism was 120 to 135 mg. per cent—definitely low. He makes the statement that increased water content, increased water binding of tissues, decreased serum cholesterol, and lowering of immunity go hand in hand.

Robinson, Brain and Kay (1927) found that in the preconvulsive period in epileptics the cholesterol level, both in blood and plasma, fell and the seizures took place at or almost at the lowest point in the curve. They mention that it was low at the menstrual period, which corresponds to the times of most frequent epileptic attacks. Gosden, Fox and Brain (1929) report that the average cholesterol level in epilepsy was subnormal and that it was more variable at the time of the seizure than at other times. Gray and McGee (1932) found that with whole blood cholesterol in normals at 194 mg. per cent, in epileptics it averaged 165 mg. per cent and in feeble-minded persons, 154 mg. per cent.

Since phospholipid and cholesterol are believed to constitute a balanced physiological pair, it may well be that it is the phospholipid-to-cholesterol ratio which influences the passage of water from the blood. Actually, as noted by McQuarrie, Husted and Bloor (1933), epileptic seizures were correlated with a high phospholipid to cholesterol ratio in the blood rather than a low cholesterol. They studied the plasma lipids (phospholipid, cholesterol, fat) throughout the day in eight severely epileptic children. No constant relationship was observed between the lipid levels and the occurrence of seizures, but in these and other cases studied, the phospholipid to cholesterol ratio tended to be higher near the time of seizures than at other times.

Skin disorders

Eczema. Hansen (1937), inspired by the work of Burr on the fat-deficiency disease, found that the iodine number of the fatty acids of the blood of children with eczema was lower than that of normal children and also that the eczema could be improved by feeding linseed oil which brought the iodine number back to within normal limits. Since that time, much work has been done on the degree of unsaturation of the blood fatty

acids in disease, but without fruitful results (Bloor, Blake and Bullen, 1938).

Acne. Strickler and Adams (1932) could find no relation between blood cholesterol and acne vulgaris in any stage.

Infections

Infections and immunity

The relation of cholesterol to immunity has been investigated and the findings indicate that blood cholesterol is concerned in the reaction of the body to infectious disease. If cholesterol is concerned in the immunization process, a change in blood cholesterol would be expected at the onset of the disease, together with a compensatory change at convalescence, and the data available indicate that both take place.

Chauffard, Laroche and Grigaut (1911) came to the conclusion that hypocholesterolemia is the rule during infections with fever and that the lowering is proportional to the shock of infection. Achard, Grigaut, Le Blanc and David (1928) reported that cholesterol and lecithin both fall at the onset of infectious fevers and on convalescence rise again to normal values. Stern (1920) found low cholesterol values at the onset of scarlet fever, rising gradually to normal in convalescence. In the tertiary benign and in the febrile quaternary form of malaria, the blood cholesterol was above normal, whereas in the apyretic quaternary form it was lower (D'Alessandro, 1931). Shope (1930) found that during the incubation period of cholera in the hog the cholesterol was low. With the onset of symptoms the values were above normal for 4-7 days, then below normal for 8-11 days. Okey and Boyden (1927) found that even mild infections, such as a common cold, will cause a noticeable fall in blood cholesterol. A number of other investigators who have studied the blood lipids in fever found either no change or a decrease (Hamano, 1931; Raab, 1933; Boyd, Orr and Reed, 1936). Marino (1933) could find constant and considerable changes in the blood lipids as the result of infections only in typhoid fever, in which cholesterol, cholesterol esters, and phospholipids were below normal during the febrile period and rose to figures above normal during convalescence. The change in cholesterol esters was greatest, and this fact led him to the conclusion that they play an important part in the defense mechanism against toxins. Knauer (1928b) found in convalescence after infections that there was often an increase to considerably beyond the normal blood level—a compensatory hypercholesterolemia.

McQuarrie and Stoesser (1932) found that the fall of cholesterol in infections, as noted above, was accompanied by a fall in phospholipid and total fatty acid to definitely subnormal values which returned to normal

during convalescence. The total leucocyte count tended to vary inversely with the lipid content of the plasma. That elevated body temperature of itself was not responsible for the changes was indicated by the fact that fever, artificially produced by phenylethylhydantoin, failed to have any such effect.

In infectious diseases, the desaturating power of the organism on the fatty acids appears to be lowered, since the iodine number of the blood lipids falls on infection and rises only slowly on convalescence.

The characteristic fall of cholesterol in infections, as well as in menstruation, affects chiefly the ester fraction; and since the fatty acids in ester combination have the highest iodine number of the blood fatty acids, the fall in iodine number of the blood fatty acids would be expected to accompany the fall in cholesterol esters. Stoesser (1940) found that the decrease in cholesterol was in the cholesterol ester fraction, that the degree of unsaturation of the plasma fatty acids was below normal in infections, and that in pneumonia the rapid fall in iodine value was followed by a rapid rise. Boyd (1935b) reported a sudden influx of unsaturated fatty acids into the blood at the onset of fever.

Leupold and Bogendörfer (1922) found that feeding cholesterol to animals increased their resistance to infection. Tunnicliff (1923) found, both *in vitro* and *in vivo*, that the addition of cholesterol increased the opsonic and cytophagic indices in blood up to double.

Raab's (1929) results in dogs show that fever from vaccines or tetrahydro-β-naphthylamine, also cooling with water, and hunger, caused a marked increase in the free fat (Bang's method—petroleum ether fraction), from which he concludes that fever metabolism is essentially hunger metabolism.

Complete lipid studies of precipitates from horse and rabbit Type I antipneumococcic serums and the serums themselves have been made by Horsfall and Goodner (1936). The lipid patterns of horse and rabbit antiserums were practically identical, but the specific precipitates differ in that the one from the horse serum appears to contain cephalin, that from rabbit serum lecithin.

Sperry and Stoyanoff (1934) found the following as the result of infection of rats with *Salmonella danysz*: (1) decrease of combined cholesterol in the liver and (2) increase of free cholesterol in the entire rat. Cholesterol-fed animals showed a large increase in the combined cholesterol in the liver over those which had not been fed cholesterol.

Serological reactions

Very little is known about the relation of the lipids to the serological reactions, although there are indications that research in this field would

be profitable. For example, the "antigens" for detection of syphilis by the Wassermann and Kahn procedures are largely lipid in nature, containing varying proportions of phospholipid and cholesterol, and a few examinations made in this laboratory of the Kahn precipitate collected from positive syphilis tests showed it to consist of about equal parts of alcohol-insoluble material, probably protein, and lipid, which consisted of equal parts of cholesterol and phospholipid.

Craig and Williams (1921) found that the feeding of cholesterol to rabbits resulted in enormous accumulations in the blood which persisted for several days, but there was no relationship between the cholesterol content of the blood serum and the Wassermann reaction. All the animals experimented with gave a constantly negative reaction despite the enormous increase of cholesterol in the blood.

Knudson, Ordway and Ferguson (1921) found that blood from syphilitic patients and from rabbits showing a four plus Wassermann reaction had a normal content of total cholesterol but considerably lower content of cholesterol esters. Workers in general are agreed that the Wassermann test for syphilis is independent of the amount of cholesterol in the blood (Knudson, Ordway and Ferguson, 1921; Craig and Williams, 1921).

Koldaev (1921) found no difference in the cholesterol content of the serum of normal horses and horses immunized to tetanus, diphtheria, and typhoid. Velluz (1933) could find no difference in the cholesterol-cholesterol ester distribution between normal and immune serum. Rywosch (1922) fed white rats on cholesterol and beef fat for a long time with the result that their erythrocytes became abnormally sensitive to lysis by water, but relatively insensitive to saponin. Sperry and Stoyanoff (1935) found that feeding cholesterol had no effect on resistance to infection with *Salmonella danysz.*

Tuberculosis

Henning (1922) found cholesterol uniformly low in tuberculosis blood when determined colorimetrically after saponification, but normal when determined without saponification, indicating the presence of a relatively large amount of cholesterol-like substance which was altered by the saponification. Total fatty acids and lecithin were normal, but free fat was high. Sweany (1924) found that the cholesterol esters increased to three times normal in healing fibroid tuberculosis and decreased to one-half in unfavorable and terminal cases. Levinson and Petersen (1923) found increased blood lipids in early pulmonary tuberculosis and decreased values in severe cases. King and Bruger (1935) found that a maintained low level of blood cholesterol (below 150 mg. per cent) signified an early fatal termination.

Leprosy

Paras (1931) found total plasma lipids very low and cholesterol slightly reduced in leprosy. Treatment with chaulmoogra oil caused a return to normal values. Low values for cholesterol were also found by Boyd and Roy (1928). The results of Villela, Castro and Anderson (1936) show almost the opposite: high total lipids with low iodine number. The cholesterol, however, was low.

Syphilis

Feigl (1918d) reported very high values for blood lecithin in aortitis luica. Knauer found the blood cholesterol in sero-negative lues to be normal, in sero-positive, generally lower than normal. Rosen and Krasnow (1932) did not find abnormal values for either cholesterol or phospholipid in syphilis, although phospholipid tended to be high and cholesterol low; the latter rose, however, as the disease progressed.

Mental Disease

Feigl (1918b) found high lecithin in 50 per cent of his cases (tabes, paralysis, taboparalysis). Fat and cholesterol increased together, but the increases in fat were greater. Cholesterol was high in one-third of his cases. In dementia praecox, Gibbs (1925) found low values for blood cholesterol. Stenberg (1929) found in manic-depressive insanity that the cholesterol values were above normal. In dementia praecox, there was increased cholesterol in the emotionally heightened and lowered cholesterol in the emotionally dulled patients.

Organic Diseases

Abnormal conditions which are not caused primarily by nutritional difficulties or definite infections are discussed here. They include diseases of the heart and circulation, the liver, and the kidneys, as well as glandular disorders.

Angina pectoris

Davis, Stern, and Lesnick (1937) reported a higher level of total and free cholesterol, phospholipid and total fatty acids in angina pectoris than in normal individuals.

Arteriosclerosis and hypertension

Moderate hypercholesterolemia was found in arteriosclerosis by Blix (1926), Schmidt (1914), Gorham and Meyers (1917), Denis (1917), and Labbe and Heitz (1922). Labbe and Heitz (1922) found that high blood pressure is generally accompanied by hypercholesterolemia, and there is

likely to be high blood cholesterol in arteriosclerosis, even when the blood pressure is low. Wacker and Fahrig (1932) found all the lipids of blood serum high in essential hypertension. In diabetics with severe arteriosclerosis, the percentage of cholesterol in plasma combined as ester has been found definitely high: 70 per cent as against the normal 60 per cent (Gibbs, Buckner and Bloor, 1933); and there is a growing suspicion that there is a relationship between the high cholesterol esters of the blood and the accumulation of these substances in the arterial walls, which is the first stage in the production of arteriosclerosis. Holden (1937) and Page, Kirk, and Van Slyke (1936) could find no evidence of a relation between blood pressure and plasma cholesterol saturation in a series of cases which included malignant and benign hypertension and chronic hemorrhagic nephritis. The plasma was approximately saturated with cholesterol in all cases. Medvei (1932) did not find supersaturation in hypertension, although the blood was saturated. Davis, Stern and Lesnick (1937) found higher values for cholesterol, lipid phosphorus and total fatty acids in hypertension, a finding which was not confirmed by Elliot and Nuzum (1936), who did not find high cholesterol in uncomplicated hypertension even when vascular degeneration or renal impairment accompanied the hypertension. Higher values were found in underweight than in obese persons. Bürger and Möbius (1934) could find no increase in cholesterol.

The relation of injury of the vessel walls to the deposition of cholesterol and cholesterol esters was investigated in rabbits by Duff (1936). Histologic changes always preceded the deposition, especially in the aortic media. Injured areas in the aortic wall became impregnated with lipids more rapidly and abundantly than other parts of the aorta. Eberhard (1936) found that alcohol in the diet, while it raised the blood cholesterol of rabbits, decreased its tendency to deposit in the arterial walls. Landé and Sperry (1936) could find no correlation between the cholesterol content of the blood and the degree of atherosclerosis in humans. In confirmation of the work of others, Page and Bernhard (1935) found that organic iodine compounds prevented the deposition of cholesterol in the vessel walls of rabbits receiving cholesterol in olive oil. The lipemia was more marked and persistent in the animals receiving organic iodine than in those not receiving it, and the lipemia differed from that of human beings with nephrosis in containing higher concentrations of total, free, and ester cholesterol as compared with other lipids, especially the phospholipids. Their opinion was that a third factor—the receptivity of the tissues—was important in determining the deposition of lipids in tissues. This significant suggestion is similar in its conception to that of the

removal mechanism offered as an explanation of the lipemia of diabetes (p. 167).

Arthritis

The total cholesterol content of plasma tends to be decreased in rheumatoid arthritis and increased in osteoarthritis. The ratio of free to bound cholesterol is normal (Hartung and Bruger, 1935).

Liver disease

The role of the liver in cholesterol metabolism is not well understood, but the investigations to date make it plain that a normal liver and a normal flow of bile are important for the formation of cholesterol esters and the preservation of their level in the blood. The bile is one path of escape for cholesterol from the blood, but not the only one. Because of the blocking of this path of excretion for cholesterol, there is frequently a high blood cholesterol in jaundice; but because there are other paths, there is not always a rise and the height of blood cholesterol is not a good measure of the severity of the disease. According to Knauer (1928b), phospholipid frequently changes more in jaundice than does cholesterol. Gardner and Gainsborough (1930), as the result of a study of blood cholesterol in hepatic and biliary disease, found that there were two types of change in cholesterol metabolism in these cases. In obstructive jaundice or complete external fistula (in both cases, with absence of bile from the intestine) the free cholesterol of the blood remained the same, but the esterified cholesterol fell. If there was complete obstruction for a long time, hypercholesterolemia resulted, but the percentage of cholesterol in the esterified form remained below 50 (normal 60-70 per cent). In uncomplicated cholelithiasis, no high blood cholesterols were encountered, and in cases where there was high blood cholesterol, *e.g.*, nephrosis and pregnancy, there was no cholelithiasis, so that no relation could be established between hypercholesterolemia and gallstones. These authors think that there was also no relation between the amount of cholesterol in the food and the occurrence of gall stones, because there was an adequate path of excretion. Furthermore, in individuals who cannot excrete cholesterol (Schoenheimer, 1933), the excess cholesterol does not appear as gallstones but in the form of nodules in the skin and joints. In other words, gallstones are formed from cholesterol already excreted, and their formation is the result of conditions in the bile itself or in the bile passages.

Mancke (1931) found in simple icterus a marked hypercholesterolemia with normal ester concentration. In subacute atrophy of the liver, the cholesterol was high and no esters were present. In cirrhosis of the liver,

the values were normal. In diabetic xanthomatosis, the cholesterol level was high.

Epstein (1931) investigated in particular the cholesterol ester partition in the plasma in liver diseases. He found that the percentage of cholesterol ester in plasma ran parallel to the severity of liver damage as shown by clinical condition and tests, almost disappearing in severe damage and increasing with improvement. The ability to esterify was lost in acute parenchymatous liver injury and was in general sensitive and easily disturbed. In chronic and slowly developing conditions, the liver may retain or regain this power. The relation between these findings and those of Gardner and Gainsborough is not entirely clear except that in both cases, there may be a cessation of the flow of bile into the intestine, and hence a lack of bile salts which have been found necessary for cholesterol absorption and esterification. Both are agreed that a normally functioning liver is essential for maintaining a normal level of cholesterol esters in the blood. On the other hand, damage to the liver by chloroform, carbon tetrachloride, or phosphorus according to Nakatsuka (1931) produced little effect on the blood lipids of rabbits. Ligation of the bile ducts produced lipemia. In parenchymatous hepatic disease, with decrease in the ratio of ester to total cholesterol in the blood there was found a 35-49 per cent decrease in the concentration of plasma total lipid, total fatty acid, total and ester cholesterol, and phospholipid (Boyd and Connell, 1938).

Feigl (1918a) found increased cholesterol and fat but decreased lecithin in acute yellow atrophy. Cholesterol esters first decreased, then rose premortally. The lipid content of the erythrocytes was not much changed.

Under normal conditions, the bile fistula dog excretes 0.5-1.0 mg. of cholesterol per kilo per day. Feeding cholesterol, either as such or as egg yolk or brain, raises the excretion slightly but the increase amounts to only about 0.1 per cent of the amount fed. Bile salt along with cholesterol increases the excretion markedly (60 mg. per 24 hours) (Wright and Whipple, 1934). High blood cholesterol with dissociation of the normal ratio of esterified to total cholesterol was found in chronic liver injury due to chloroform. It also developed after chronic biliary obstruction, in which case, however, it could be reduced to below normal by chloroform poisoning or bile duct infection. Acute chloroform poisoning, inadequate food consumption, or fasting did not change blood cholesterol. Absence of bile in the intestine resulting in faulty fat absorption did not alter the blood cholesterol or its ester ratio (Hawkins and Wright, 1934). Liver damage due to carbon tetrachloride was found to retard the removal from the blood of injected fat (Nachlas, Duff, Tidwell and Holt, 1936).

Kidney disorders

Nephritis. The reports on the blood lipids in nephritis found in the literature are conflicting, a fact which may be partly due to failure to distinguish between kidney disease due to destructive changes in the glomeruli and that due to changes in the tubules. Thus Denis (1917) found notably high cholesterol in only one case out of fifty of the various types examined. Similarly, Bloor (1917), in a series of cases, found only the fat of the blood above normal, the cholesterol and lecithin being normal or below. Lecithin was occasionally high in the corpuscles in fatal cases just before death. On the other hand, Epstein and Rothschild (1917) reported very high values: cholesterol up to 1.23 grams per 100 cc. of blood in chronic parenchymatous nephritis, whereas in uremic cases very low values for cholesterol were found.

Earlier workers often found very high values for blood lipids in nephritis. Thus Müller (1913) gave the following values for the lipemic blood taken postmortem from a case of nephritis: total ether extract 3.6 per cent, neutral fat 2.15, cholesterol 0.84, lecithin 0.69. Nephritis was one of the conditions in which lipemia was observed in the days of blood-letting (Fischer, 1903). It is necessary to distinguish between the two main types of nephritis in considering blood cholesterol. Parenchymatous nephritis, or nephrosis, is much more likely to result in abnormal blood lipids than the interstitial type. In the parenchymatous type, or nephrosis, the high lipids appear to be the result of a generalized tissue disturbance with cellular breakdown. In the interstitial type of disease, abnormality of the blood lipids is much less common and may perhaps be referred to the occurrence of acidosis.

In the interstitial type, Byrom and Kay (1927) report slightly low values for lipid phosphorus in nephritic blood. Work by Hiller, Linder, Lundsgaard and Van Slyke (1924) brought out the following with regard to the blood lipids in nephritis: Determinations of the plasma lipids and of the respiratory quotient and total metabolism (Tissot method) performed with nephritic and normal subjects before and after ingested fat in doses of 1 gram per kilo body weight showed that after fat ingestion, a greater increase of fatty acids and lecithin was present in the plasma of nephritics with initially high blood lipids than in the plasma of normal subjects or of nephritics without lipemia. In cholesterol, no differences were found. The nephritic patients with lipemia were, however, able to burn fat as efficiently as normal individuals and the accumulation of fat in their blood may have been due to a disturbance of the mechanism for transferring lipids from the blood to the tissue deposits. Maxwell (1928) found renal edema associated with increased blood cholesterol. In

recovery, the edema disappeared first; then the cholesterol fell to normal. King and Bruger (1935) reported that renal amyloid disease with proteinuria was accompanied by a progressive increase in cholesterol.

Nephrosis. The phenomenon of high cholesterol in the blood in nephrosis has been studied by several workers. Macheboeuf and Sandor (1931) found that in normal serum, 1 gram of albumin was combined with about 70 mg. of lipid. In nephrotic serum, 1 gram of albumin carried 700 to 800 mg. They thought that the fatty substances might play a role, small but not negligible, in maintaining the colloidal osmotic pressure of the blood, and agreed with Fishberg that the increase of fatty substances in nephrosis may be useful in contributing to the dynamic equilibrium of salts and water between plasma and tissues by its effect on the Donnan equilibrium.

Calvin and Goldberg (1931) concluded that the cholesterol content of the blood during nephrotic edema was higher than normal and might remain so after the edema disappeared. Cholesterol values varied with the intensity of the edema and the cholesterol changes were preceded by the edema.

Lichtenstein and Epstein (1931) report that the cholesterol ester of the blood in nephrosis was often markedly above the normal. The total cholesterol as usual was very high and the phospholipid high, but not often excessive. They found that thyroxin greatly increased the blood cholesterol, a finding which was not corroborated by Bonilla and Moya (1931).

The hypothesis of Fishberg that the blood lipids, and especially the cholesterol, can act vicariously for the diminished protein in maintaining the colloidal osmotic pressure of plasma in nephrosis was commented on by Rabinowitch (1930). He pointed out that the edema of this condition might be due to other causes produced by the lipids, for example, changes in imbibition pressure or altered membrane permeability in the kidneys. He sought to apply the hypothesis to conditions in the blood in diabetes where the blood pressure is often high, and which is thought by some to contribute to arteriosclerosis. He found that the protein content of plasma in diabetic hypercholesterolemia was normal or higher, and that the albumin-to-globulin ratio was normal. Stasiak (1924) produced a hypercholesterolemia in the experimental nephritis of uranium nitrate, mercuric chloride, or cantharidin. He thought the high values were due to general lipoid degeneration produced by the poisons. Maxwell (1928) regarded the high cholesterol and edema of nephrosis as the result of a toxic process. Bing and Starup (1935) could find no relation between the hyperlipemia and the hypoproteinemia. There was a relationship between the cholesterol and protein excretion, but not a constant one.

The urine cholesterol did not depend on the blood cholesterol concentration or on diuresis. Cowie, Jarvis and Cooperstock (1930) found that a blood protein of 5 per cent or more was necessary to keep a patient free from edema. This was accomplished by a protein intake producing a nitrogen balance of +1 to +3.76 grams. Cholesterol in this individual was about 550 mg. per cent. Port (1932) found a rough correspondence between the blood cholesterol content and the edema in the nephrotic individuals. The edema first appeared with a cholesterol content of 400-500 mg. per cent.

It is significant that experimental damage of the kidney by uranium poisoning (Politzer, 1936) produced a marked increase in all the blood lipids: neutral fat in the proportion of 1:3.3; free cholesterol, 1:2.3; phosphatides and total fatty acids, 1:1.77; and cholesterol esters, 1:1.1.

The changes in blood lipids in interstitial nephritis are thus neither marked nor characteristic. In nephrosis, they are often both marked and characteristic. No satisfactory explanation can be offered for the differences.

Pancreatitis

Very few data are available on pancreatitis. Brunner (1935) found no change in two cases, increased ester concentration in one, and profound changes in the lipid economy of the fourth: total cholesterol increased seven-fold, ester cholesterol twelve-fold. The carbohydrate metabolism was also disturbed: Insulin therapy improved the lipid condition before the carbohydrate metabolism was affected.

Thyroid conditions

Knauer (1928b) summed up the then available facts as follows: In hyperthyroidism there is a decrease of blood lipids, in hypothyroidism an increase in both phospholipid and cholesterol; and this summary holds today. Artom (1923, 1924) found in thyroidectomized rabbits a decrease of phospholipid and an increase of cholesterol. Schmidt and Hughes (1938) found that experimental hyperthyroidism did not produce significant changes in the cholesterol of the blood of dogs. Thyroidectomy produced a marked increase of both free and bound cholesterol which was reduced to normal levels by the administration of thyroxin without disturbing the normal ratio of free to bound, and without hypocholesterolemia. They believe that the hypocholesterolemia sometimes found in clinical hyperthyroidism may be due to factors other than increased thyroid activity. Westra and Kunde (1933) found that the high cholesterol in the blood of cretin rabbits could be lowered by feeding thyroid. Boyd (1936) reported that subtotal thyroidectomy in man produced an

increase in all plasma lipids but no change in corpuscle values. The increase was greatest for fat, then free cholesterol, then ester cholesterol, and phospholipid least. The cause was believed to be a diminished rate of uptake of fat by the tissues. Mason, Hunt, and Hurxthal (1930) found that the cholesterol content of blood was decreased in hyperthyroidism and was lowest in acute toxemia. It was markedly increased in true myxedema. Nichols and Perlzweig (1928) found a decrease in blood lipids in hyperthyroid cases. Treatment with iodine and operation resulted in a rise of total fat, together with a fall in iodine number. In severe hyperthyroidism, the cholesterol was low, in agreement with the findings of most other workers. The findings of all workers agree, therefore, that the level of the blood lipids varies with the activity of the thyroid.

A differentiation from changes in basal metabolic rate was shown by Cutting, Rytand and Tainter (1934) by the use of dinitrophenol. This drug raised the basal metabolic rate without affecting the blood cholesterol. A relation of blood cholesterol to basic metabolic rate was indicated by the work of Gilligan and associates (1934).

Hypophysis dysfunction

Studies on hypophysis dysfunction are few. Raab (1926) found that extracts of the mid and hind portions of the hypophysis caused lowering of blood fat. According to Coope and Chamberlain (1925), pituitrin caused fat accumulation in rabbit livers up to twice the normal value. Knauer (1928b) reported very low values for cholesterol (44 mg. per cent) and phospholipid (70 mg. per cent) in a child of three years suffering from dystrophia adiposogenitalis, the result of defective functioning of the pituitary.

Operative procedures

Castration. In 40 men castrated according to Danish law, Teilum (1937) found a gradual increase in cholesterol for at least five years. The average for the fifth to ninth year after the operation was 247 mg. per cent with a maximum of 306 mg. per cent. Age was not a factor.

Splenectomy. In dogs, neither Dubin and Pearce (1916) nor Marino (1931) was able to produce definite effects on the blood lipids by removal of the spleen. In rats, according to Randles and Knudson (1926), removal of the spleen or suprarenals did not affect the cholesterol level.

Hepatectomy and ligation of the bile duct. In rats, Chanutin and Ludewig (1936) found a marked decrease in plasma cholesterol esters on the first day after partial hepatectomy, with a return to normal on the second or third day. There was an increase of free cholesterol on the second day. In animals with ligated bile ducts, there was an increase

in free cholesterol. Lipid phosphorus varied directly with the free cholesterol.

Emotional stimulation and sympathectomy. Emotional stimulation, which is known to raise blood sugar, has also been shown (Lyons, 1931) to produce increases in blood cholesterol of 25 to 30 per cent in cats, the values returning to normal within an hour. Sympathectomized animals did not respond and the effect of the bile was excluded.

Effect of the Lipids of the Blood on Its Properties

Lipemia

Lipemia, sometimes called hyperlipemia, is a condition of abnormally high content of lipids in blood plasma, usually associated with a milky appearance of the plasma. It occurs under various circumstances and is apparently the result of a failure to remove fat from the blood. The accumulated material consists mainly of fat and contains considerable amounts of phospholipids and cholesterol esters, together with free cholesterol (Marble and Smith, 1936; Bloor, 1921b).

High blood lipids without visible milkiness have been reported from time to time. Thus, in addition to earlier references, Bloor (1921b) found, in a diabetic, values up to 4.35 per cent total lipid with clear plasma. The lipid solubility was apparently an unstable state because the clear plasma, when left standing overnight, was milky in the morning. Allen (1923) reported a case of hepatic cirrhosis with a total fat value of 3.63 and a clear plasma. Bürger (1922) found in cases of mechanical icterus a latent cholemic lipemia with increase of fat values from the normal of 0.4-0.5 per cent to a value of 2.2 per cent with clear serum. The increases included the lipids other than fat, and increase in cholesterol preceded the increase in glycerides. With continuance of bile occlusion, the proportion of cholesterol in the ester form diminished.

Embolism

The fat in the blood is ordinarily in a form so finely divided that it passes through the blood vessels without difficulty. Occasionally fat may be present in the blood in particles large enough to plug some of the smaller vessels, producing embolism. A review of the literature on this subject is given by Groskloss (1935). Fat embolism occurs most frequently in the lungs, then in the heart, and least frequently in the brain.

Hemolysis

Sueyoshi and Okawa (1929) found that fresh lecithin and cephalin do not cause hemolysis but do so when rancid, and that this activity is counteracted by cholesterol, as is also the hemolysis caused by oleic acid.

Since lysolecithins and lysocephalins are strongly hemolytic (Levene and Rolf, 1924; Belfanti, 1925); the hemolysis may have been caused by partial hydrolysis of the phospholipids in the course of the development of rancidity. Washed red cells are resistant to hemolysis by hypotonic salt solutions, but when lecithin or oleic acid is added the sensitivity is restored. Grigaut, Debray and Furstner (1925) found that the resistance of the red blood corpuscles to hemolysis increased with their cholesterol content.

Coagulation

That certain of the lipids, especially cephalin, are concerned in blood coagulation has been believed for a long time. The large amount of work on the subject, including his own, has been reviewed by Howell (1935). Cephalin from various organs, especially the spleen, was found by De Barbieri (1932) to accelerate the coagulation of recalcified oxalated plasma. It was possible, by removal of cephalin, to prevent recoagulation of oxalated plasma, and, by the addition of cephalin, to restore coagulability. He believed that the increase in coagulability of blood after bleeding was due to an increase of cephalin in the circulation. Fischer and Hecht (1934), in reviewing the literature on the subject, raise the doubt as to whether pure cephalin is an active coagulant, referring to the efforts of Gratia and Levene (1922) to prepare a pure cephalin of high coagulative activity. Later, Chargaff (1937) reported that the cerebrosides, and also a sphingomyelin, from animal brains were active inhibitors of coagulation. Cerebroside sulfuric acid was found to be an anticoagulant.

Sedimentation time of corpuscles

Westergren, Theorell and Widström (1931), in a study of 84 clinical cases, showed a strong correlation between fibrin and sedimentation rate, a weak correlation for globulin, none for albumin, and none for lecithin and cholesterol. By removal of the lipids from blood proteins by extraction without denaturization, it has been shown that the plasma lipids have no effect on the suspension stability of the blood corpuscles (Ohlson and Rundquist, 1932). Forced fattening was found to increase the sedimentation rate in pigs (Knauer, 1928a). Theorell (1930) found that cholesterol added to blood strongly reduced the sedimentation rate of red blood corpuscles. There was no strict proportionality between the lipid content and the suspension stability.

Physico-chemical relations

From a review by Theorell (1930), the following may be summarized. Cholesterol lowers the surface tension of water markedly, lecithin less

markedly. Cholesterol slows the sinking of the red blood corpuscles greatly, lecithin much less so. Colloidal cholesterol is a neutral body with a lower dielectric constant than water, and has a negative charge. Lecithin at the plasma pH is amphoteric, with the isoelectric point far on the acid side, and has therefore a negative charge. Both lecithin and cholesterol collect at the corpuscle-plasma interfaces. There should thus be a relation between lipid content and suspension stability, which is not the case.

Combinations of the lipids with other substances in blood

Protein. Evidence regarding cholesterol-protein combinations has been supplied from several sources, and combinations between phospholipid and protein are frequently suggested. The constituent of the blood with which they are most closely related appears to be the euglobulin fraction. Handovsky and co-workers (1925) found that the higher the plasma euglobulin the less cholesterol could be extracted by prolonged shaking with ether. Eufinger (1928) found the same relation between high euglobulin and cholesterol-binding in pregnancy. In the blood of non-pregnant women, 90 per cent of the cholesterol could be extracted by three hours' shaking with ether, but in pregnant women at the time of parturition, only 63 per cent could be so extracted. The euglobulin fraction increased correspondingly as pregnancy proceeded. Gardner and Gainsborough (1927) obtained the following figures for combination of cholesterol and protein in blood: percentage of sterol retained by total proteins, 16.4 per cent; by the euglobulin, 13.8 per cent; pseudoglobulin, 1.03 per cent; albumin, 1.5 per cent. The ability of blood serum to dissolve cholesterol has been examined by Eck and Desbordes (1934) in aged persons. In twenty-five samples, with cholesterol varying from 123 to 575 mg. per cent, it was found that only four could dissolve more cholesterol, and that seventeen deposited cholesterol on standing, *i.e.*, were supersaturated. They relate the power to dissolve and hold cholesterol to the protein composition of the serum.

Troensegaard and Koudahl (1926) present evidence that the cholesterol is combined with the blood globulin through the same carbon atom that holds the hydroxyl group. Theorell (1930) adds the observation that at the isoelectric point of the globulin all the cholesterol can be extracted with ether. Compounds containing protein, phospholipid, and cholesterol have been prepared by Macheboeuf (1929) from blood plasma. Such a protein fraction, very rich in lecithin and cholesterol esters, was soluble in water to a clear solution containing up to 5 per cent. Macheboeuf and Sandor (1932) found that ether will not extract a notable amount of lipid from serum. Addition of alcohol (5-13 per cent) results

in a 50 per cent extraction. The lipids extractable in this way are those attached to the globulin; the albumin compounds remain resistant to extraction. Turner and Gibson (1932) studied the lipids carried down by precipitation of the blood proteins of human and dog plasma and horse serum and found that proteins carried down about one-half of the total lipids, the globulins removing the larger amounts. Enough has been done to show that the combination of the blood protein, especially the globulin, with the lipids is probably extensive and important, and that this type of combination would explain much of the characteristic behavior of the lipids in blood, for example, their apparent solubility in the face of the fact that notably cholesterol is practically insoluble in water or salt solution. Pryzlecki and Hofer (1936) showed experimentally that on mixing proteins with lecithin, especially with oleic acid, precipitates are obtained containing large amounts of lipid. The precipitation depends on the pH and the lipid can be extracted from the precipitate by alcohol, except in the case of the lecithin-albumin combination, which is resistant.

Chloride. Peters and Man (1934) found that a small amount of chlorine was dissolved in the petroleum ether extract of normal blood and larger amounts in that of patients with nephrosis. Christensen and Corley (1938) confirm their results and suggest that it is the result of entrainment of the halide with the phospholipid during extraction, possibly a loose binding with the zwitterion phospholipid.

Carbon dioxide. Van Slyke, Sendroy, Hastings and Neill (1928) found that salts or proteins decreased the solubility of carbon dioxide in blood but that lipids increased it. The solubility of carbon dioxide in serum is ordinarily 93-94 per cent of its solubility in water. In lipemic serum, the solubility may be higher than in water.

Summary

Blood lipid composition in normal animals

Corpuscles. There is general agreement that the corpuscles of warm-blooded animals are of quite constant composition, not only in individuals of the same species but in different species. In other words, corpuscles seem to behave like tissue cells in having a characteristic composition.

Plasma. The lipid content of the plasma varies considerably in composition from animal to animal, even of the same species, and is still more variable from species to species. There is some variation in the same animal at different times, but less than between different animals of the same species. It seems to bear a definite relation to the amount of fat habitually taken in the diet. In the herbivora, in which fat is characteristically low, the plasma lipids are low, while in all animals whose food

contains considerable fat there is found a higher content of cholesterol and phospholipid in plasma. A reasonable inference is that these two lipids are concerned with the transport of fat in the plasma and probably with its metabolic changes in the tissues.

Abnormal bodily states

Corpuscles. As noted above, the corpuscles tend to preserve their characteristic lipid composition. The only condition in which this composition has been found to be altered is in the new corpuscles formed as the result of severe hemorrhage. In these, the lipid content is high, as it is in young cells in general.

Plasma. Definite variations in the lipid content of plasma are found in infections in which there is certainly a fall in blood cholesterol and probably in phospholipid as well; in menstruation, where there is a fall in cholesterol esters; and in diabetes where the blood lipids are high, or at least on the high side of normality. The plasma lipids are characteristically high in essential xanthomatosis and in nephrosis. The fall in cholesterol in infections may be connected with the immunity processes, since the level comes back to normal again on recovery. The high values in diabetes may probably be referred to the large use of fat, which would bring about an increase both in phospholipid and cholesterol. In xanthomatosis, the cholesterol is high, due to a failure in excretion. In nephrosis, the reason for the high lipids is unknown. Increased blood cholesterol in this condition is thought by some to be a vicarious effort to supplement the low plasma proteins in their effect on colloidal osmotic pressure.

The shifts in plasma cholesterol esters are especially notable. Their amount and percentage fall in menstruation and in liver infections (due to failure in formation). They are high both in percentage and total amount in xanthomatosis (up to 90 per cent of the total cholesterol), in magnesium deficiency (85 to 90 per cent of the total), in nephrosis, vitamin B_1 deficiency, and in severe arteriosclerosis with gangrene. During the absorption of cholesterol, their percentage of the total cholesterol is adjusted to the percentage found in blood plasma by the formation of cholesterol esters during the absorption, which takes place only with the simultaneous absorption of fat and with the aid of bile. Cholesterol esters deposit in tissues which, if not actually degenerating, have a low level of metabolism.

Bibliography

Abderhalden, E., "Physiological Chemistry," trans. by W. T Hall and G Defren, New York, J. Wiley and Sons, 1911 p. 122, 123
Abelous, J. E. and Soula, L. C., *Compt. rend. soc. biol.*, 85: 6 (1921). p. 143
Achard, A., Grigaut, A., Le Blanc, A. and David, M., *J. physiol. path. gén.*, 26: 415 (1928). p. 172

Adams, P. D., *see* Strickler, A.
Allen, F. M., *J. Metabolic Research*, **2**: 219 (1922). p. 166, 168
ibid., **3**: 775 (1923). p. 183
Allen, F. M. and Wishart, M. B., *J. Metabolic Research*, **4**: 613 (1923). p. 145
Anderson, J. Van D., *see* Villela, G. G.
Anderson, R. J., *J. Biol. Chem.*, **71**: 407 (1926). p. 116, 122
Arnoldi, W. and Collazo, I. A., *Z. ges. exptl. Med.*, **40**: 323 (1924). p. 170
Artom, C., *Arch. intern. physiol.*, **22**: 173 (1923); **23**: 394 (1924) p. 181
Artom, C. and Peretti, G., *Boll. soc. ital. biol. sper.*, **7**: 980 (1932); *C. A.*, **27**: 342 (1933). p. 139
Asada, K., *Biochem. Z.*, **142**: 44 (1923). p. 163
Avrin, I., *see* Erickson, B. N.
Bang, I., *Biochem. Z.*, **90**: 383 (1918a). p. 120, 129
ibid., **91**: 104 (1918b). p. 120, 138, 139, 140, 154
ibid., **91**: 111 (1918c). p. 137, 143
ibid., **94**: 359 (1919). p. 139
Bantin, C. F., *J. Am. Med. Assoc.*, **86**: 546 (1926). p. 166
Barjot, G., *see* Leroy, A.
Baumann, E. J., *see* Underhill, F. P.
Belfanti, S., *Z. Immunitäts.*, **44**: 347 (1925). p. 184
Benedict, S. R., Osterberg, E. and Neuwirth, I., *J. Biol. Chem.*, **34**: 217 (1918). p. 121
Benotti, J., *see* Thannhauser, S. J.
Bernardbeig, J., *see* Rémond, A.
Bernhard, A., *see* Leopold, J. S.
Bernhard, W. G., *see* Page, I. H.
Beumer, H., *Z. Kinderheilk.*, **35**: 298 (1923a); *Z. ges. exptl. Med.*, **35**: 328 (1923b); *Klin. Wochschr.*, **6**: 1749 (1927); *ibid.*, **7**: 758 (1928). p. 142
See also Bürger, M.
Bidwell, E. H., *see* Shillito, F. H.
Binet, L., *see* Roger, H.
Bing, J. and Starup, U., *Acta Med. Scand.*, **86**: 12 (1935). p. 180
Blake, A. G., *see* Bloor, W. R.
Blatherwick, N. R., *J. Biol. Chem.*, **49**: 193 (1921). p. 121, 165
Blix, G., *Acta Med. Scand.*, **64**: 142, 175, 234 (1926) p. 121, 138, 140, 151, 165, 175
Bloor, W. R., *J. Biol. Chem.*, **19**: 1 (1914) p. 119, 120, 130, 131, 132, 137, 154, 155, 156
ibid., **23**: 317 (1915). p. 125, 127, 130, 138, 139
ibid., **24**: 447 (1916a). p. 120, 130
ibid., **25**: 577 (1916b). p. 129, 133
ibid., **26**: 417 (1916c). p. 125
ibid., **31**: 575 (1917) p. 179
Bull. soc. chim. biol., **3**: 451 (1921a). p. 125
J. Biol. Chem., **49**: 201 (1921b). p. 183
ibid., **56**: 711 (1923). p. 117, 126, 131, 159, 161
ibid., **59**: 543 (1924); **63**: 1 (1925). p. 117, 159, 161
ibid., **95**: 633 (1932). p. 146
ibid., **103**: 699 (1933). p. 131, 132
See also Gibbs, C. B. F., McQuarrie, I., Snider, R. H., Sundstroem, E. S.
Bloor, W. R. and Knudson, A., *J. Biol. Chem.*, **27**: 107 (1916). p. 129
Bloor, W. R. and MacPherson, D. J., *J. Biol. Chem.*, **31**: 79 (1917). p. 160
Bloor, W. R., Blake, A. G. and Bullen, S. S., *J. Allergy*, **9**: 227 (1938). p. 117, 172
Bloor, W. R., Gillette, E. M. and James, M. S., *J. Biol. Chem.*, **75**: 61 (1927). p. 130, 138, 139, 141, 165, 166
Blotner, H., *Arch. Internal Med.*, **55**: 121 (1935). p. 138
Blumgart, H. L., *see* Gilligan, D. R.
Bodansky, M., *Proc. Soc. Exptl. Biol. Med.*, **28**: 630 (1931). p. 127, 139
Bogendörfer, L., *see* Leupold, E.
Boggs, T. R. and Morris, R. S., *J. Exptl. Med.*, **11**: 553 (1909). p. 158
Bondi, S. and Neumann, A., *Wien. klin. Wochschr.*, **23**: 734 (1910). p. 118
Bonilla, E. and Moya, A., *Endokrinologie*, **9**: 171 (1931). p. 180
Bosch, G., *see* Collazo, J. A.
Bose, J. P. and De, U. N., *Indian J. Med. Research*, **24**: 489 (1936). p. 135
Boyd, E. M., *J. Biol. Chem.*, **101**: 323 (1933a). p. 129, 133, 134
ibid., **101**: 623 (1933b). p. 128, 133
J. Clin. Investigation, **13**: 347 (1934). p. 149
J. Biol. Chem., **110**: 61 (1935a). p. 135
Can. Med. Assoc. J., **32**: 500 (1935b). p. 173
J. Biol. Chem., **115**: 37 (1936). p. 127, 181
Endocrinol., **21**: 292 (1937). p. 149
Boyd, E. M. and Connell, W. F., *Arch. Internal Med.*, **61**: 755 (1938). p. 178
Boyd, E. M. and Stevenson, J. W., *J. Biol. Chem.*, **122**: 147 (1937). p. 127, 159
Boyd, E. M. and Tweddell, H. J., *Trans. Roy. Soc. Can.*, **29**: 113 (1935). p. 139
Boyd, E. M. and Wilson, K. M., *J. Clin. Investigation*, **14**: 7 (1935). p. 150
Boyd, E. M., Orr, J. H. and Reed, G. B., *Proc. Soc. Exptl. Biol. Med.*, **35**: 479 (1936). p. 172
Boyd, G. L., *Am. J. Diseases Children*, **36**: 298 (1928). p. 165
Boyd, T. C. and Roy, A. C., *Indian J. Med. Research*, **15**: 643 (1928). p. 175
Boyden, R. E., *see* Okey, R.
Braeuning, H., *see* Neisser, E.
Brain, W. R., *see* Gosden, M., Robinson, S. H. G.
Bruger, M., *see* Hartung, E. F., King, S. E.
Bruger, M. and Poindexter, C. A., *Arch. Internal Med.*, **53**: 423 (1934) p. 170
Bruger, M. and Somach, I., *J. Biol. Chem.*, **97**: 23 (1932). p. 134
Brun, G. C., *Biochem. Z.*, **287**: 420 (1936). p. 133
"Cholesterol Content of the Red Blood Cells in Man," trans by H. Anderson, Copenhagen, Arnold Busck, 1939. p. 127
Brunner, W., *Klin. Wochschr.*, **14**: 1853 (1935). p. 181

Buchwald, K. W., *see* Mattick, W L
Buckner, E., *see* Gibbs, C. B. F.
Bürger, M., *Münch. med. Wochschr.*, **69**: 103 (1922). p. 183
Ergeb inn. Med. Kinderheilk., **34**: 583 (1928). p. 139
Bürger, M. and Beumer, H., *Biochem. Z.*, **56**: 446 (1913). p. 128
Bürger, M. and Möbius, W., *Klin. Wochschr.*, **13**: 1349 (1934). p. 176
Bugnard, L., *Compt. rend. soc. biol.*, **102**: 369 (1929). p. 143
Bull. soc. chim. biol., **12**: 98 (1930). p. 143
Bulger, H. A., Smith, F. M. and Steinmeyer, A., *J. Am. Med. Assoc.*, **91**: 1964 (1928). p 169
Bullen, S. S., *see* Bloor, W. R.
Burgheim, —, *Klin. Wochschr.*, **6**: 1354 (1927). p. 162
Burns, F. H., *see* Riddle, O.
Burt, M. L., *see* Page, I. H.
Byrom, F. B. and Kay, H. D., *Brit. J. Exptl. Path.*, **8**: 429 (1927). p. 133, 179
Calvin, J. K. and Goldberg, A. H., *Am. J. Diseases Children*, **41**: 1066 (1931). p. 180
Cashin, M. F. and Moravek, B., *Am. J. Physiol.*, **82**: 294 (1927). p. 145
Castro, A., *see* Villela, G. G.
Cattoretti, F., *Arch. ital. biol.*, **63**: 113 (1915). p. 157
Chaikoff, I. L., *see* Lorenz, F. W.
Chaikoff, I L. and Kaplan, A., *J. Biol. Chem.*, **106**: 267 (1934); **112**: 155 (1935). p. 167
Chaikoff, I. L., McGavack, T H. and Kaplan, A., *J. Clin. Investigation*, **13**: 1 (1934). p. 142
Chamberlain, E. N., *J. Physiol.*, **68**: 259 (1929). p. 149
See also Coope, R.
Chamberlain, E. N. and Corlett, R. L., *Brit. J. Exptl Path.*, **13**: 299 (1932). p. 159
Chambers, W. H., *see* Himwich, H. E.
Channon, H. J. and Collinson, G. A., *Biochem J.*, **23**: 1212 (1929). p. 117
Chanutin, A. and Ludewig, S., *J. Biol. Chem.*, **115**: 1 (1936). p. 182
J. Lab. Clin. Med., **22**: 903 (1937). p. 169
Chargaff, E., *J Biol. Chem.*, **121**: 175 (1937). p. 184
Chase, L. A., *Can Med. Assoc. J*, **17**: 197 (1927). p. 165
Chauffard, A., Laroche, G. and Grigaut, A, *Semaine méd.*, **31**: 577 (1911) p. 172
Ann méd, **8**: 69 (1920). p. 120, 139
Ching, T. T., *see* Rony, H. R.
Christensen, H. N. and Corley, R. C., *J. Biol. Chem*, **123**: 129 (1938). p. 186
Church, A. E, *see* Sure, B.
Cohn, D. J., *see* Gordon, M. B
Collazo, I. A., *see* Arnoldi, W.
Collazo, J. A and Bosch, G., *Biochem. Z.*, **141**: 370 (1923). p. 163
Collinson, G. A., *see* Channon, H. J
Colombies, H., *see* Rémond, A.
Connell, W. F., *see* Boyd, E. M.
Coope, R. and Chamberlain, E. N., *J. Physiol*, **60**: 69 (1925). p 149, 182
Coope, R. and Mottram, V. H., *J. Physiol*, **49**: 23 (1914). p. 148
Cooperstock, M., *see* Cowie, D M.
Corcoran, A. C. and Rabinowitch, I. M, *Biochem J*, **31**: 343 (1937). p. 135
Corlett, R L., *see* Chamberlain, E. N.
Corley, R. C., *see* Christensen, H. N.
Cowie, D. M. and Hoag, L. A., *J. Am Med Assoc*, **77**: 1493 (1921). p. 140, 151
Cowie, D M., Jarvis, K. M. and Cooperstock, M, *Am. J. Diseases Children*, **40**: 465 (1930) p. 181
Craig, C F and Williams, W. C., *Am J. Syphilis*, **5**. 392 (1921). p. 174
Cramer, W., *J. Physiol.*, **54**: ii (1920). p 163
Csonka, F. A, *J. Biol. Chem.*, **33**: 401 (1918) p 159
Curtis, A. C., Sheldon, J. M. and Eckstein, H. C., *Am. J. Med Sci*, **186**: 548 (1933). p. 167
Cutting, W C., Rytand, D. A. and Tainter, M L., *J. Clin. Investigation*, **13**: 547 (1934). p. 182
Daddi, L., *Sperimentale*, **52**: 43 (1898). p. 154
D'Alessandro, R., *Arch. farmacol. sper.*, **52**: 258 (1931). p. 172
Dannenberg, F., *Biochem. Z.*, **244**: 128 (1932). p. 162
David, M, *see* Achard, A.
Davis, D., *see* Gilligan, D. R.
Davis, D., Stern, B. and Lesnick, G., *Ann. Internal Med.*, **11**: 354 (1937). p. 175, 176
De, U. N., *see* Bose, J. P.
De Barbieri, A., *Boll. soc. ital. biol. sper.*, **7**: 489 (1932); *C. A.*, **26**: 5633 (1932). p. 184
Debray, M., *see* Grigaut, A.
Degkwitz, R., *Ergeb. Physiol.*, **32**: 821 (1931). p. 129
Denis, W., *J. Biol. Chem.*, **29**: 93 (1917). p. 175, 179
Derlin, L., *see* Neisser, E.
Desbordes, J., *see* Eck, M.
De Voss, G., *Z. physiol. Chem.*, **205**: 20 (1932). p. 162
Downes, H. R. and Pack, G. T., *Am. J. Cancer*, **16**: 290 (1932). p. 162
Dubin, H., *J. Biol. Chem.*, **33**: 377 (1918). p. 160
Dubin, H. and Pearce, R. M., *Arch. Internal Med.*, **18**: 426 (1916). p. 182
Ducceschi, V., *Arch. ital. biol.*, **70**: 93 (1920); *C. A.*, **15**: 898 (1921). p 156
Duff, G. L., *Arch. Path.*, **22**: 161 (1936). p. 176
See also Nachlas, A.
Dunlop, D. M., *see* Stewart, C. P.
du Vigneaud, V. and Karr, W. G., *J. Biol. Chem.*, **66**: 281 (1925). p. 121
Eberhard, T. P., *Arch. Path.*, **21**: 616 (1936). p. 176
Eck, M. and Desbordes, J., *Compt. rend. soc. biol.*, **117**: 428 (1934). p. 185
Eckstein, H. C., *J. Biol. Chem.*, **62**: 737 (1925). p. 136
See also Curtis, A. C.
Elliot, A. H. and Nuzum, F. R, *Arch. Internal Med.*, **57**: 63 (1936). p 176
Ellis, G. W. and Gardner, J. A., *Proc. Roy. Soc. London*, **B85**: 385 (1912). p. 155
Entenman, C., *see* Lorenz, F. W.
Epstein, A. A. and Rothschild, M. A., *J. Biol. Chem.*, **29**: iv (1917). p. 179

Epstein, E. Z., Arch. Internal Med., 47: 82 (1931). p. 178
See also Lichtenstein, L.
Erdmann, R., see Kaufmann, C.
Erickson, B. N., Williams, H. H., Avrin, I. and Lee, P., J. Clin. Investigation, 18: 81 (1939). p. 128
Eufinger, H., Arch. Gynäk., 133: 475 (1928). p 149, 185
Fahrig, C., see Wacker, L.
Farr, L. E., see Page, I. H.
Feigl, J , Biochem. Z , 86: 1 (1918a). p 178
ibid., 88: 53 (1918b). p. 175
ibid., 90: 361 (1918c). p. 133
ibid., 92: 1 (1918d). p. 165, 175
ibid., 92: 282 (1918e). p. 157
ibid., 115: 63 (1921). p. 160, 161
Ferguson, H., see Knudson, A.
Fetterman, J. L. and Kumin, H. J., J. Am. Med. Assoc., 100: 1005 (1933). p. 171
Fischer, A. and Hecht, E., Biochem. Z., 269: 115 (1934) p 184
Fischer, B., Arch. path. Anat. (Virchow's), 172: 30 (1903). p. 164, 166, 179
Fish, P. A., see Gage, S. H.
Fishberg, E. H., J. Biol. Chem., 81: 205 (1929). p. 159, 162
Fonseca, F , Deut. med Wochschr., 50: 362 (1924). p. 167
Fox, J. T., see Gosden, M.
Freudenberg, E., Biochem. Z., 45: 467 (1912). p. 155
Freyberg, R. H., Newburgh, L. H. and Murrill, W. A , Arch. Internal Med , 58: 589 (1936). p. 165
Friedenthal, H., see Munk, I.
Furstner, W E., see Grigaut, A.
Gabbe, E , Münch. med. Wochschr., 68: 1377 (1921). p 144
Gaddie, R., see Stewart, C. P.
Gage, S. H. and Fish, P. A , Am. J. Anat., 34: 1 (1924) p 136, 140, 153, 154
Gainsborough, H., see Gardner, J. A.
Gardner, J. A , Biochem. J., 18: 777 (1924). p. 146
See also Ellis, G. W.
Gardner, J. A. and Gainsborough, H , Biochem. J., 21: 141 (1927) p 185
Quart. J. Med., 23: 465 (1930). p 177
Gardner, J A., Gainsborough, H. and Murray, R., Biochem J , 32· 1457 (1938) p 135
Gibbs, C. B. F., Buckner, E and Bloor, W R., New Engl J Med. Surg , 209 384 (1933) p 176
Gibbs, C. E., Am. J Psychiatry, 5: 189 (1925). p. 175
Gibson, R. B., see Turner, M. E
Gilbert, M and coworkers, J Biol. Chem., 67: 629; 69· 9 (1926). p 121
Gildea, E. F., see Man, E. B.
Gillette, E. M., see Bloor, W. R.
Gilligan, D. R. and associates (Volk, M. C , Davis, D and Blumgart, H. L.), Arch Internal Med , 54: 746 (1934). p. 182
Gilmour, C. R., see McEachern, J. M.
Goldberg, A. H., see Calvin, J K
Goodner, K., see Horsfall, F. L., Jr.
Gordon, M. B. and Cohn, D J , Am J Diseases Children, 35· 193 (1928). p 152
Gorham, F. D and Meyers, V. C , Arch. Internal Med , 20· 599 (1917) p 175
Gosden, M., Fox, J T. and Brain, W. R., Lancet, 2: 12 (1929). p. 171
Gratia, A. and Levene, P. A., J. Biol Chem., 50: 455 (1922) p. 184
Gray, H., Am. J. Med. Sci., 168. 35 (1924). p. 165
Gray, H. and McGee, L. C., Arch. Neurol. Psychiat , 28: 357 (1932). p 171
Gray, M. S., see Kaiser, A D
Green, H H. and Macaskill, E H , J. Agr. Sci., 18: 384 (1928); C. A , 23: 2207 (1929) p 152
Greenwald, I., J. Biol Chem , 21: 29 (1915). p 133
Grigaut, A., see Achard, A., Chauffard, A.
Grigaut, A. and Yovanovitch, R , Compt rend. soc. biol., 91: 1310 (1924). p 129
Grigaut, A., Debray, M. and Furstner, W E., Compt. rend soc biol , 92. 935 (1925) p 184
Groskloss, H. H., Yale J. Biol. Med., 8: 59 (1935). p 183
Guthmann, H., Arch. Gynäk , 140: 202 (1930). p. 162
György, P., Jahrb. Kinderheilk., 112: 283 (1926). p. 150, 151
Hamano, H., Proc. Imp Acad. (Tokyo), 7: 80 (1931). p. 172
Handovsky, H. and coworkers, Arch. ges. Physiol. (Pflügers), 210: 59, 63 (1925) p 185
Hanner, J. P., see Wilson, W. R.
Hansen, A. E., Am. J. Diseases Children, 53: 933 (1937). p. 171
See also Wilson, W. R
Hansen, A. E , Wilson, W. R. and Williams, H. H., J. Biol. Chem., 114: 209 (1936). p. 146
Hartung, E. F. and Bruger, M., J. Lab. Clin. Med., 20: 675 (1935). p 177
Hastings, A. B., see Van Slyke, D. D.
Haustein, F., Arch. Kinderheilk., 86: 33 (1929). p. 171
Hawkins, W. B. and Wright, A., J. Exptl. Med., 59: 427 (1934). p 178
Hecht, E., see Fischer, A.
Heitz, J., see Labbe, M.
Hellmuth, K., Arch. Gynäkol., 127: 293 (1926). p. 150
Henning, B. H., J. Biol. Chem., 53: 167 (1922). p. 174
Herbert, F. K., Biochem. J., 29: 1887 (1935). p. 165
Herrmann, E. and Neumann, J., Biochem. Z., 43: 47 (1912). p. 149
Hiller, A., Linder, G. C., Lundsgaard, C. and Van Slyke, D. D., J. Exptl. Med , 39: 931 (1924). p. 138, 140, 141, 179
Himwich, H. E. and associates (Chambers, W. H., Hunter, A. L. and Spiers, M. A.), Am. J. Physiol., 99: 619 (1932). p 168
Hiraoka, T., see Schmitz, E.
Hoag, L. A., see Cowie, D. M.
Hofer, E., see Pryzlecki, St. J. v.
Holden, R. F., Jr., J. Clin. Investigation, 16: 763 (1937). p. 176

Holt, L. E., Jr, *see* Nachlas, A

Holt, L. E., Jr., Tidwell, H. C. and Scott, T. F. M, *J. Pediatrics*, **6**: 151 (1935). p. 144

Horiuchi, Y., *J. Biol. Chem*, **44**: 345 (1920a). p. 124, 125, 129, 158

ibid., **44**: 363 (1920b). p 158

Horsfall, F. L., Jr and Goodner, K, *J Exptl Med*, **64**: 855 (1936). p. 173

Hotta, S, *Tôhoku J Exptl Med*, **16**. 311 (1930). p. 140

Howell, W. H., *Physiol Revs*, **15**: 435 (1935) p 184

Howland, J. and Richards, A. N, *J. Exptl. Med*, **11**: 344 (1909) p. 156

Hueck, W. and Wacker, L., *Biochem Z.*, **100**. 84 (1919) p 139

Hughes, H. B., *see* Schmidt, L H.

Hunt, H. M, *New Engl. J Med.*, **201**: 659 (1929). p 145

See also Mason, R L., White, P.

Hunter, A. L., *see* Himwich, H. E.

Huntsinger, M. E., *see* McClure, C. W.

Hurxthal, L. M., *see* Mason, R. L.

Husted, C., *see* McQuarrie, I.

Imrie, C. G., *J. Biol Chem*, **20** 87 (1915) p 164, 166

Iscovesco, H, *Compt rend soc biol.*, **72**: 985 (1912). p 126

Iwatsuru, R., *Arch ges Physiol.* (*Pflügers*), **202**: 194 (1924). p 126, 163

James, M. S, *see* Bloor, W R

Jarvis, K. M., *see* Cowie, D M

Kaiser, A. D. and Gray, M S., *Am J. Diseases Children*, **47**. 9 (1934). p. 152

Kaplan, A., *see* Chaikoff, I L

Karr, W. G., *see* du Vigneaud, V., Oser, B. L.

Kashiwabara, M., *see* Yamakawa, S.

Kaufmann, C. and Erdmann, R, *Biochem Z*, **249** 438 (1932) p 148, 162

Kaufmann, C. and Mublbock, O, *Arch Gynäk*, **136**: 478 (1929). p. 147

Kay, H. D., *see* Byrom, F. B., Robinson, S H G.

Kik, M C., *see* Sure, B

King, S. E and Bruger, M., *Ann. Internal Med*, **8**: 1427 (1935). p 174, 180

Kirk, E, *see* Page, I. H

Klein, R. I and Levinson, S A, *Proc Soc. Exptl Biol Med*, **31**: 353 (1933). p. 145

Klemperer, G and Umber, H, *Z klin. Med*, **61** 145 (1907) p 164

Knauer, H., *Z. physiol. Chem*, **176**: 151 (1928a). p 170, 184

Jahrb. Kinderheilk., Abhandl., **22** (1928b) p 149, 161, 172, 177, 181, 182

Knudson, A., *J. Biol. Chem*, **32**: 337 (1917). p 120, 127

ibid., **45**: 255 (1921). p 139

See also Bloor, W. R, Randles, F S

Knudson, A., Ordway, T. and Ferguson, H, *Proc Soc. Exptl Biol Med*, **18**: 299 (1921). p. 174

Koch, F., *see* Schmitz, E.

Koldaev, B. M, *Russian J. Physiol.*, **3**. 139 (1921); *C A*, **17** 2138 (1923) p 174

Kooy, R. and Rosenthal, W., *Acta Brevia Neerland Physiol.*, **3**: 185 (1933); *C. A.*, **28**: 2770 (1934). p. 138, 145

Koudahl, B., *see* Troensegaard, N

Kramer, B., *J. Exptl. Med.*, **17**: 206 (1913). p. 156

Krasnow, F, *see* Rosen, I

Kruse, H. D., Orent, E. R. and McCollum, E V., *J. Biol. Chem.*, **100**: 603 (1933). p. 164

Kultjugin, A., *Biochem. Z*, **186**. 36 (1927). p. 164

Kumin, H. J., *see* Fetterman, J. L.

Kunde, M. M, *see* Westra, J. J.

Labbe, M and Heitz, J., *Compt. rend soc biol*, **87**: 1024 (1922). p. 175

Landé, K. E. and Sperry, W. M., *Arch. Path*, **22**. 301 (1936). p 176

Laroche, G., *see* Chauffard, A.

Lattes, L., *Arch. exptl. Path. Pharmakol.*, **66**: 132 (1911). p. 130, 155, 156, 168

Laudat, M., *see* Widal, F.

Lawrence, J. V. and Riddle, O., *Am. J. Physiol.*, **41**: 430 (1916). p 148

Le Blanc, A., *see* Achard, A.

Le Breton, E., *Bull. soc. chim. biol.*, **3**: 539 (1921). p. 122

Lecoq, R., *Bull. soc. chim. biol.*, **15**: 1498 (1933). p. 164

See also Leroy, A.

Lee, P., *see* Erickson, B. N.

Leites, S. and associates, *Z. ges. exptl. Med.*, **90**: 378 (1933); *Klin. Wochschr.*, **13**: 1056 (1934). p. 142, 144

Leopold, J. S., Bernhard, A. and Tow, A., *Am. J. Diseases Children*, **43**: 882 (1932). p. 164

Leroy, A. and associates (Lecoq, R., Veline, M, Vallissant, Mme. and Barjot, G.), *Lait*, **11**: 12, 144, 234, 359 (1931); *C. A.*, **25**: 5454 (1931). p. 151

Lesnick, G., *see* Davis, D.

Leupold, E. and Bogendörfer, L., *Deut. Arch. klin. Med.*, **140**: 28 (1922). p. 173

Levene, P. A., *see* Gratia, A.

Levene, P. A. and Rolf, I. P., *J. Biol. Chem.*, **60**: 677 (1924). p. 184

Levinson, S. A., *see* Klein, R. I.

Levinson, S. A. and Petersen, W. F., *Am Rev. Tuberc.*, **7**: 278 (1923). p. 174

Levy, A. J., *see* Rony, H. R.

Lewin, A. I., *Z. ges. exptl. Med.*, **96**: 532 (1935). p. 168

Lichtenberg, H. H., *see* Schwarz, H.

Lichtenstein, L. and Epstein, E. Z., *Arch. Internal Med.*, **47**: 122 (1931). p. 180

Limberger, W. A., *see* Wilson, G.

Linder, G. C., *see* Hiller, A.

Ling, S. M., *Chinese J. Physiol*, **5**: 381 (1931). p. 145

Long, C. N. H. and Venning, E. M., *J. Biol. Chem.*, **96**: 397 (1932). p. 153

Lorenz, F. W., Chaikoff, I. L. and Entenman, C., *J. Biol. Chem.*, **126**: 763 (1938). p. 148

Lorenz, F. W., Entenman, C. and Chaikoff, I. L., *J. Biol Chem*, **122**: 619 (1938). p. 148

Ludewig, S., *see* Chanutin, A.

Ludlum, S. D., Taft, A. E. and Nugent, R. L., *Colloid Symposium Annual*, **7**: 233 (1929). p. 136

Lundsgaard, C., *see* Hiller, A.
Lyons, C., *Am. J. Physiol.*, **98**: 156 (1931) p. 183
MacAdam, W. and Shiskin, C., *Quart. J. Med.*, **16**: 193 (1923). p. 161
McArthur, E. H., *J. Biol Chem.*, **87**: 299 (1930). p. 140
Macaskill, E. H., *see* Green, H. H.
McCarrison, R., *Indian J. Med. Research*, **6**. 550 (1919) p. 163
McCay, C. M., *see* Maynard, L. A.
McClure, C. W. and Huntsinger, M. E., *J. Biol. Chem.*, **76**: 1 (1928). p. 134, 144
McCollum, E. V., *see* Kruse, H. D.
McEachern, J. M. and Gilmour, C. R., *Can. Med. Assoc. J.*, **26**: 30 (1932). p. 134
McGavack, T. H., *see* Chaikoff, I. L.
McGee, L. C., *see* Gray, H.
Macheboeuf, M. A., *Bull. soc. chim. biol*, **11**: 268 (1929). p. 185
Macheboeuf, M. A. and Sandor, G., *Bull. soc. chim biol*, **13**: 745 (1931). p. 180
ibid., **14**: 1168 (1932). p. 185
MacPherson, D. J., *see* Bloor, W. R.
McQuarrie, I., *Am J. Diseases Children*, **38**. 451 (1929). p. 171
McQuarrie, I and Stoesser, A V., *Proc. Soc. Exptl. Biol. Med.*, **29**: 1281 (1932). p. 172
McQuarrie, I., Husted, C and Bloor, W R., *J. Clin Investigation*, **12**: 255 (1933). p 171
Macy, I G and associates, *J Biol Chem*, **118**: 599 (1937). p. 161
ibid., **122**: 515 (1938). p. 128
Mahler, A., *J. Biol Chem.*, **69**: 653 (1926). p. 157
Man, E. B., *see* Peters, J. P
Man, E. B. and Gildea, E F., *J. Biol Chem*, **99**: 61 (1932). p. 137, 139, 140
J. Clin. Investigation, **15**: 203 (1936). p. 155
J Biol. Chem, **119**: 769 (1937) p. 133, 135
Mancke, R., *Deut. Arch. klin. Med*, **170**: 358 (1931). p. 177
Mann, F C., *see* Markowitz, C.
Mansfeld, G., *Arch. ges Physiol (Pflügers)*, **129**: 46 (1909). p. 169
Marble, A. and Smith, R M., *Arch. Ophthalmol.*, N S., **15**: 86 (1936). p. 183
Marino, S., *Arch. farmacol sper.*, **52**. 73 (1931). p. 182
ibid., **55** 1 (1933). p 172
Markowitz, C and Mann, F C, *Am J. Physiol.*, **93**: 521 (1930). p. 143
Marsh, P L. and Waller, H. G, *Arch. Internal Med.*, **31**. 63 (1923). p. 165
Mason, R. L., Hunt, H. M. and Hurxthal, L M., *New Eng. J Med*, **203**: 1273 (1930) p. 182
Mattick, W. L. and Buchwald, K W., *J Cancer Research*, **12**: 236 (1928); **13**: 157 (1929) p. 162
Maxwell, J., *Quart. J. Med.*, **21**: 297 (1928). p. 179, 180
Mayer, A. and Schaeffer, G, *J. physiol path gén*, **15**: 984 (1913). p. 120, 124, 131
Compt. rend. acad. sci, **159**: 102 (1914). p. 152
Maynard, L A. and McCay, C. M., *J. Nutrition*, **2**: 67 (1929). p. 146
Medvei, C V, *Klin. Wochschr.*, **11**: 414 (1932). p. 176
Meyers, V C., *see* Gorham, F. D.
Milbradt, W., *Biochem. Z.*, **223**: 278 (1930). p. 138, 159, 163
Mills, E. S, *see* Rabinowitch, I. M
Mjassnikow, A L, *Z. klin Med.*, **103**. 767 (1926). p 145
Mobius, W., *see* Bürger, M.
Molitch, M. and Poliakoff, S., *Arch. Pediat*, **53**: 613 (1936). p. 152
Moravek, B., *see* Cashin, M F
Morgulis, S. and Spencer, H C., *J. Nutrition*, **12**: 173 (1936). p 170
Morris, R S, *see* Boggs, T. R
Mosenthal, H O, *Arch Internal Med.*, **50**: 684 (1932). p 140
Mottram, V. H, *see* Coope, R.
Moya, A., *see* Bonilla, E
Mühlbock, O., *see* Kaufmann, C.
Müller, J, *Z. physiol. Chem.*, **86**: 469 (1913). p. 179
Muller, G. L., *Am. J Med. Sci.*, **179**: 316 (1930). p. 161
Muller, G. L. and Talbot, J. H., *Arch. Internal Med.*, **47**: 855 (1931). p. 134
Munk, I., *Ergeb. Physiol.*, **1**: 296 (1902). p. 127
Munk, I. and Friedenthal, H., *Zentr. Physiol.*, **15**: 297 (1901). p. 139
Murlin, J R. and Riche, J. A., *Am. J. Physiol.*, **40**: 146 (1916). p. 152
Murray, R., *see* Gardner, J. A.
Murrill, W. A, *see* Freyberg, R. H.
Nachlas, A., Duff, G. L., Tidwell, H. C. and Holt, L. E., Jr., *J. Clin. Investigation*, **15**: 143 (1936). p 178
Nakatsuka, M., *Japan. J. Gastroenterol.*, **3**: 153 (1931). p. 178
Neill, J. M., *see* Van Slyke, D. D..
Neisser, E. and Braeuning, H., *Z. exptl. Path. Therap.*, **4**: 747 (1907). p. 136, 140
Neisser, E. and Derlin, L., *Z. klin. Med.*, **51**: 428 (1904). p. 165
Nerking, J., *Münch. med. Wochschr.*, **56**: 1475 (1909). p. 156
Neumann, A., *Wien. klin. Wochschr.*, **20**: 851 (1907). p. 136, 140
See also Bondi, S.
Neumann, J., *see* Herrmann, E.
Neuwirth, I., *see* Benedict, S. R.
Newburgh, L. H, *see* Freyberg, R. H.
Nichols, E. G. and Perlzweig, W. A., *J. Clin. Investigation*, **5**: 195 (1928). p. 182
Nissen, N. I., *Acta Med. Scand*, **73**: 99 (1930). p. 170
Nomura, T., *Tôhoku J. Exptl. Med.*, **12**: 247 (1929). p. 144
Nugent, R L., *see* Ludlum, S. D.
Nuzum, F. R., *see* Elliot, A. H.
Ohata, S., *J. Biochem* (Japan), **16**: 191 (1932). p. 164
Ohlson, B. and Rundquist, O., *Biochem. Z.*, **247**: 249 (1932). p. 184
Okawa, K, *see* Sueyoshi, Y.
Okey, R. and Boyden, R. E., *J. Biol. Chem.*, **72**: 261 (1927). p. 147, 172
Onohara, K., *Biochem. Z.*, **163**: 67 (1925). p. 163
Ordway, T., *see* Knudson, A.

Orent, E. R., *see* Kruse, H. D
Orr, J. H., *see* Boyd, E. M.
Oser, B. L. and Karr, W. G , *Arch. Internal Med* , **36**: 507 (1925). p. 133
Oshima, T., *Zentr. Physiol.*, **21**: 297 (1907). p 148
Osterberg, E., *see* Benedict, S R.
Pack, G. T., *see* Downes, H R
Page, I. H., *see* Pasternak, L.
Page, I. H. and Bernhard, W. G., *Arch. Path.*, **19**: 530 (1935). p. 176
Page, I. H., Farr, L. E. and Weech, A. A , *J. Biol. Chem* , **121**: 111 (1937). p. 147
Page, I. H., Kirk, E. and Van Slyke, D D., *J. Clin. Investigation*, **15**: 101 (1936). p. 176
Page, I. H., Pasternak, L. and Burt, M. L., *Biochem. Z* , **223**: 445 (1930). p. 140
ibid., **231**: 113 (1931). p. 168
Paras, E. M., *J. Philippine Is. Med Assoc* , **11**: 1 (1931); *C A* , **26**: 766 (1932) p 175
Parhon, C. J. and Parhon, M., *Compt rend soc. biol* , **88**: 231 (1923a). p 148, 151
ibid., **89**: 349 (1923b); **90**: 150 (1924). p. 148, 151
Pasternak, L., *see* Page, I. H.
Pasternak, L. and Page, I. H., *Biochem. Z.*, **252**: 254 (1932). p 144
Patrzek, F., *see* Rosenthal, F.
Patterson, J. W. T , *Biochem J.*, **21**: 958 (1927). p 153
Pearce, R. M., *see* Dubin, H
Peretti, G., *see* Artom, C.
Perlzweig, W. A., *see* Nichols, E. G
Peters, J. P. and Man, E. B., *J Biol. Chem.*, **107**: 23 (1934). p 186
Petersen, W. F., *see* Levinson, S. A.
Pfeiffer, G , *Biochem. Z* , **220**: 210 (1930). p. 127
Plass, E. D. and Tompkins, E. H., *J Biol. Chem* , **56**. 309 (1923) p 150
Poindexter, C. A , *see* Bruger, M
Poliakoff, S., *see* Molitch, M.
Politzer, M., *Arch farmacol. sper* , **62**: 70 (1936); *C. A.*, **31**: 1495 (1937). p. 181
Port, F., *Deut Arch klin. Med* , **174**: 312 (1932). p 181
Pryzlecki, St. J. v. and Hofer, E., *Biochem. Z.*, **288**: 303 (1936). p. 186
Raab, W., *Z ges. exptl Med.*, **53**: 317 (1926). p. 182
Wien. Arch inn Med , **18** 387 (1929). p. 173
Z. ges. exptl. Med., **89**: 616 (1933) p 172
Rabinowitch, I. M., *Arch. Internal Med.*, **46**: 752 (1930). p 180
See also Corcoran, A. C.
Rabinowitch, I. M and Mills, E. S , *J. Metabolic Research*, **7**: 87 (1925). p. 167
Radsma, W., *Geneesk Tijdschr Nederland -Indië*, **69**: 793 (1929). p. 135
Randles, F. S. and Knudson, A., *J. Biol. Chem.*, **67**: xvii (1926). p. 182
Reed, G. B., *see* Boyd, E M.
Reicher, K., *Z klin. Med.*, **65**: 235 (1908). p 156
Verhandl. deut. Kong. inn. Med., **28**: 327 (1911). p. 138, 139
Rémond, A., Colombies, H. and Bernardbeig, J., *Compt. rend. soc. biol.*, **90**: 1029 (1924). p. 142
Richards, A. N., *see* Howland, J.
Riche, J. A., *see* Murlin, J R
Riddle, O., *see* Lawrence, J V.
Riddle, O. and Burns, F H , *Am. J. Physiol* , **81**: 711 (1927). p 148
Robinson, S. H G., Brain, W. R and Kay, H. D., *Lancet*, **2**: 325 (1927). p. 152, 171
Roger, H. and Binet, L., *Presse méd.*, **30**: 277 (1922). p. 142
Rolf, I. P., *see* Levene, P A.
Rony, H. R. and Ching, T T., *Endocrinology*, **14**: 355 (1930) p. 121, 143
Rony, H. R. and Levy, A J , *J Lab. Clin Med.*, **15**: 221 (1929). p. 140, 170
Rose, W. C., *J Pharmacol* , **24**: 123 (1924). p 169
Rosen, I. and Krasnow, F , *Arch Dermatol Syphilol* , **26**: 48 (1932). p. 169, 175
Rosenbloom, D , *Proc. Soc Exptl Biol. Med* , **32**· 908 (1935) p 150
Rosenthal, F., *Deut. med Wochschr.*, **45**: 571 (1919). p. 147
Rosenthal, F and Patrzek, F , *Berlin klin Wochschr* , **56**: 793 (1919). p. 147
Rosenthal, W., *see* Kooy, R
Rothschild, M A , *see* Epstein, A A.
Roy, A. C., *see* Boyd, T. C.
Rundquist, O., *see* Ohlson, B
Rytand, D. A., *see* Cutting, W. C.
Rywosch, D., *Arch. ges Physiol.* (*Pflügers*), **196**. 643 (1922). p 174
Sandor, G., *see* Macheboeuf, M A.
Schaeffer, G., *see* Mayer, A.
Schaible, P. J., *J. Biol. Chem.*, **95**· 79 (1932) p. 117, 151
Schick, B., *see* Sperry, W. M.
Schmidt, H. B., *Arch Internal Med* , **13**· 121 (1914). p. 175
Schmidt, L. H. and Hughes, H B , *Endocrinology*, **22**: 474 (1938). p. 181
Schmitz, E and Hiraoka, T , *Biochem Z.*, **193**: 1 (1928). p. 163
Schmitz, E. and Koch, F., *Biochem. Z.*, **223**: 257 (1930). p. 159
Schoenheimer, R , *Z. physiol. Chem* , **180**: 1 (1929). p. 116, 143
Z. klin. Med., **123**: 749 (1933). p. 177
Schoorl, P., *Landbouwkund. Tijdschr.*, **47**: (June, 1935). p. 147, 151
Schott, E., *Deut Arch. klin. Med* , **112**: 403 (1913). p. 118
Schulz, N., *Arch. ges. Physiol.* (*Pflügers*), **65**: 299 (1897). p. 154
Schwarz, H. and Lichtenberg, H. H., *J. Biol Chem.*, **121**: 315 (1937). p. 159
Scott, T. F. M., *see* Holt, L. E., Jr.
Sendroy, J , *see* Van Slyke, D. D.
Sets, P., *see* Thannhauser, S. J.
Sheldon, J. M., *see* Curtis, A. C.
Shillito, F. H., Bidwell, E. H. and Turner, K B., *J. Biol. Chem.*, **112**: 551 (1936). p. 143
Shiskin, C., *see* MacAdam, W.

Shope, R. E., *J. Biol. Chem.*, **80**: 127 (1928a). p. 142
ibid., **80**: 133 (1928b). p. 155
ibid., **80**: 141 (1928c). p. 150
J. Exptl. Med., **51**: 179 (1930). p 172
Sinclair, R. G., *J. Biol Chem.*, **111**: 515 (1935). p. 117
ibid., **115**: 211 (1936). p. 117
Slemons, J. M. and Stander, H. J., *Bull Johns Hopkins Hosp.*, **34**: 7 (1923). p. 150
Smith, F. M., *see* Bulger, H. A.
Smith, R. M., *see* Marble, A.
Snider, R. H. and Bloor, W. R., *J. Biol. Chem.*, **99**: 555 (1933). p. 117
Somach, I., *see* Bruger, M.
Soula, L. C., *see* Abelous, J. E.
Spencer, H. C., *see* Morgulis, S.
Sperry, W. M., *J. Biol. Chem*, **111**: 467 (1935). p. 142
ibid., **114**: 125 (1936a). p. 129
Am. J. Diseases Children, **51**: 84 (1936b). p. 151
See also Landé, K. E.
Sperry, W. M. and Schick, B., *Am. J. Diseases Children*, **51**: 1372 (1936). p. 169
Sperry, W. M. and Stoyanoff, V A, *J. Biol. Chem.*, **105**: lxxxii (1934). p. 173
ibid., **9**: 131 (1935). p. 174
Spiers, M. A., *see* Himwich, H. E.
Ssokoloff, N. A., *Deut. Arch. klin Med.*, **145**: 236 (1924). p. 164
Stander, H. J., *see* Slemons, J. M.
Starup, U., *see* Bing, J.
Stasiak, A., *Biochem Z.*, **144**: 477 (1924). p. 180
Steiner, A, *see* Turner, K. B.
Steinmeyer, A., *see* Bulger, H. A.
Stenberg, S., *Acta Med. Scand*, **72**: 1 (1929). p. 175
Stern, B., *see* Davis, D.
Stern, G., *Z. Kinderheilk.*, **25**: 129 (1920). p. 172
Stevenson, J. W., *see* Boyd, E M.
Stewart, C. P., Gaddie, R and Dunlop, D M., *Biochem. J.*, **25**: 733 (1931). p 153
Stoesser, A. V., *Proc. Soc. Exptl. Biol. Med.*, **43**: 168 (1940). p. 173
See also McQuarrie, I.
Stoyanoff, V. A., *see* Sperry, W. M.
Strickler, A. and Adams, P. D., *Arch. Dermitol. Syphilol.*, **26**: 11 (1932) p. 172
Sueyoshi, Y. and Okawa, K, *Sei-i-Kwai Med. J.*, **48**, no. 9: abstracts 3 (1929); *C A*, **24**· 1902 (1930). p. 183
Sundstroem, E. S. and Bloor, W. R, *J. Biol Chem.*, **45**: 153 (1920). p. 125
Sure, B., Kik, M C. and Church, A E, *J Biol Chem*, **103**: 417 (1933). p. 155
Sweany, H C, *Am. Rev. Tuberc.*, **10**: 329 (1924). p 174
Szent-Györgyi, A. v. and Tominaga, T, *Biochem. Z.*, **146**: 226 (1924) p 161
Taft, A. E., *see* Ludlum, S. D.
Tainter, M. L, *see* Cutting, W. C.
Takahashi, K., *J. Chem. Soc. Japan*, **43**: 231 (1922) p. 164
Talbot, J. H., *see* Muller, G. L.
Tamura, M., *Arch. ges Physiol.* (*Pflügers*), **230**: 771 (1932). p. 126
Teilum, G, *Compt. rend. soc. biol*, **125**: 577 (1937). p 182
Terroine, E.-F, *Compt rend soc biol*, **76** 523 (1914a). p 132
J. physiol. path. gén., **16**: 212 (1914b). p. 118, 128
ibid., **16**: 386 (1914c). p 137, 154
"Contribution à la connaissance de la physiologie des substances grasses et lipoidiques," Paris, Masson et Cie., 1919. p 127, 128, 130, 131 132, 168
Ann. sci. nat Zool., [10], **4**: 354 (1920). p. 160
Thannhauser, S. J. and Setz, P., *J. Biol Chem*, **116**· 533 (1936). p 133
Thannhauser, S. J., Setz, P and Benotti, J, *J. Biol. Chem*, **126**: 785 (1938). p 127
Theorell, H., *Biochem. Z*, **223**: 1 (1930). p 184, 185
See also Westergren, A
Tidwell, H. C., *see* Holt, L. E, Jr., Nachlas, A.
Tolstoi, E., *J. Biol. Chem.*, **83**: 753 (1929). p. 147
Tominaga, T., *see* Szent-Györgyi, A v.
Tompkins, E. H., *see* Plass, E. D.
Tow, A., *see* Leopold, J S
Troensegaard, N. and Koudahl, B., *Z. physiol Chem*, **153**: 111 (1926) p 185
Tunnicliff, R, *J. Infectious Diseases*, **33**: 285 (1923). p. 173
Turner, K. B., *see* Shillito, F. H.
Turner, K. B. and Steiner, A, *J. Clin. Investigation*, **18**: 45 (1939). p. 138
Turner, M. E. and Gibson, R. B., *J. Clin. Investigation*, **11**: 735 (1932). p 186
Tweddell, H. J., *see* Boyd, E. M.
Tyler, M and Underhill, F. P., *J. Biol. Chem.*, **66**: 1 (1925) p 149
Umber, H., *see* Klemperer, G.
Underhill, F. P., *see* Tyler, M.
Underhill, F. P. and Baumann, E. J., *J Biol. Chem*, **27**: 169 (1916). p 155, 169
Vahlquist, B., *Biochem. J.*, **25**: 1628 (1931). p. 139
Vallissant, Mme., *see* Leroy, A.
Van de Veld, J., *Compt. rend. soc. biol*, **97**: 1825 (1928). p. 169
Van Slyke, D. D., *see* Hiller, A., Page, I. H.
Van Slyke, D. D., Sendroy, J., Hastings, A. B. and Neill, J. M., *J. Biol. Chem.*, **78**: 765 (1928). p. 186
Veline, M., *see* Leroy, A.
Vellus, L., *Compt. rend. soc. biol.*, **112**: 256 (1933). p. 174
Venning, E. M., *see* Long, C. N. H.
Versé, M., *Beitr. path. Anat.*, **63**: 789 (1917). p. 143, 146
Villela, G. G., Castro, A. and Anderson, J. Van D., *J. Trop. Med Hyg.*, **39**: 126 (1936) p. 175
Volk, M. C., *see* Gilligan, D. R.
Wacker, L., *see* Hueck, W.

Wacker, L and Fahrig, C., *Klin. Wochschr.*, 11 862 (1932) p 176
Waele, A de, *Naturw Tijdschr.*, 11. 154 (1929); *C. A.*, 24: 1672 (1930) p 126
Waksman, S. A., *Am J Physiol.*, 46. 375 (1918) p. 159
Waller, H. G., *see* Marsh. P L
Ward, K. M, *Arch. Disease Childhood*, 6. 329 (1931). p. 152
Weech, A. A., *see* Page, I. H.
Weill, A., *see* Widal, F.
Westergren, A., Theorell, H. and Widström, G., *Z ges exptl. Med*, 75· 668 (1931) p 184
Westra, J. J. and Kunde, M. M, *Am. J. Physiol*, 103· 1 (1933). p 181
Whipple, G. H., *see* Wright, A.
Whipple, G. H. and associates, *Arch. Internal Med.*, 23. 636, 689, 711 (1919) p 156
White, A. C., *Quart. J. Med.*, 19: 159 (1926). p 168
White, P. and Hunt, H, *New Engl. J. Med*, 202: 607 (1930). p 145, 170
Widal, F., Weill, A. and Laudat, M., *Semaine méd.*, 32: 529 (1912). p. 138
Widström, G., *see* Westergren, A.
Williams, H. H., *see* Erickson, B. N., Hansen, A. E
Williams, W. C, *see* Craig, C. F.
Wilson, G. and Limberger, W A., *J. Am. Med Assoc*, 101: 110 (1933). p 171
Wilson, K. M., *see* Boyd, E. M
Wilson, W R., *see* Hansen, A. E.
Wilson, W. R. and Hanner, J. P., *J. Biol. Chem*, 106 323 (1934) p. 136
Wilson, W. R. and Hansen, A. E., *J. Biol. Chem*, 112: 457 (1936) p 133
Wishart, M. B, *see* Allen, F. M.
Wright, A., *see* Hawkins, W. B.
Wright, A. and Whipple, G. H, *J. Exptl Med*, 59. 411 (1934). p 178
Yamakawa, S. and Kashiwabara, M., *Tôhoku J Exptl. Med.*, 3: 317 (1922). p. 169
Yovanovitch, R., *see* Grigaut, A.
Zucker, T. F., *Proc. Soc. Exptl. Biol. Med.*, 17: 89 (1920). p. 139

Chapter IV

The Lipids in Tissues

Introduction

The lipids which have been at all well studied in tissues are the neutral fat, the phospholipids (mainly lecithin and cephalin), and cholesterol. Of these, much work has been done only on neutral fat and cholesterol. Interest in the phospholipids as biochemical entities is quite recent, and information regarding them is in the confused early stage in which, owing to imperfect methods and limited numbers of analyses, data are often contradictory. The uncertainty is still more marked when relations between the compounds are considered, as for example between the phospholipids and cholesterol. Nevertheless, sufficient progress has been made to indicate the great importance of these compounds in cell life and activity. It is becoming increasingly clear that the lipids of tissues fall into two classes: (1) the metabolic, which serve as sources of energy, and (2) the structural or functional, which constitute essential parts of the framework and living substance of the cell. Class (1) would be expected to be easily replaceable by material coming in from the blood and gastrointestinal tract; class (2) to be fairly stable and to be replaceable only as wear and tear rendered them less useful.

Fat has the physiologically passive function of a store of concentrated foodstuff. It represents excess of intake over outgo, either originating from the fat of the food or being formed from other foodstuffs, mainly carbohydrate. The stored fat may either be used by the individual itself in times of food shortage or passed on to the offspring as fetal body lipid, or as the lipid of milk or eggs. Minor functions performed by the fats are those due to its insulating properties, against cold from without or excessive loss of heat from within; padding of joints, nerves, and organs against mechanical shocks; anchoring of organs and blood vessels; and lastly, but perhaps not the least important, supplying fatty materials for the building up of other lipids, certainly of the phospholipids and possibly of the sterols.

Each species of animals tends to store a characteristic fat which represents a balance between what it takes in or synthesizes and what it burns. The tendency to form a characteristic fat is easily affected by the fat

of the diet, so that to a very considerable extent the stored fat is the food fat more or less modified. Young animals are born with a small store of relatively hard fat—the fat which would be laid down in the relative position in the mother which the fetus occupies, according to the principles of fat deposition outlined by Henriques and Hansen (1901). After birth, the composition of the body fat changes under the influence of the fat of the milk, becoming softer and richer in olein and the glycerides of the volatile fatty acids (Bardisian, 1921). In old age (Parhon and Parhon, 1923) there is a slow but constant increase in the cholesterol in the fat stores. Wacker and Hueck (1913) found in normal human fat about 127 mg. per cent of cholesterol entirely in the free form. In pathological conditions and in old age it may be two to three times as great. Such fat is generally characterized by a deep yellow color.

The phospholipids and sterols have interested physiologists from the time of their discovery, but until recently a relatively small amount of work has been done on them because of the difficulty of working with them. At the present time, this difficulty is being overcome and data are accumulating rapidly, but information has not yet reached the stage at which much can be said with certainty as to their function. Their universal presence in living tissues in relatively constant amounts characteristic of the tissue (Bloor, Okey and Corner, 1930), and the fact that they persist in constant percentage in the tissues of animals fasted to the point of death (Terroine, 1919), all point to the probability that they are important constituents of living cells. Work showing that they are definitely connected with the absorption and transport of fat has recently been done.

As regards the sterols, Dorée (1909) summed up what was then known as follows, and not much has been added since. The protoplasm of all animals examined contains at least one member of the group, most frequently cholesterol. In warm-blooded vertebrates it is always present and is the only one found. In insects, it is replaced by an allied form. These various sterols all have the same formula ($C_{27}H_{46}O$), contain one double bond, and differ from one another only in crystal form and melting points of dibromides and acetates. The double bond and the hydroxyl group are essential for their activity. Similar substances, the phytosterols, are characteristic of plants. These differ from cholesterol in molecular weight and melting point, both of the substance itself and its acetate and dibromide.

Cholesterol never occurs in plants, and in animals it is probably not formed from plant sterols (Schoenheimer, 1931). That cholesterol takes an active part in the metabolism of the fatty acids appears from the following facts. In blood plasma, it is found esterified with the fatty acids

to the extent of about two-thirds of its total amount. During the absorption of fat and cholesterol, the cholesterol is esterified with the fatty acids to about the same extent as is found in the blood. When there is a great increase of cholesterol in the blood, as in essential xanthomatosis, the increased cholesterol is mostly in the ester form. In the fatty livers produced by cholesterol feeding, a considerable proportion of the fatty material consists of cholesterol esters.

Compounds closely related to the sterols (Sobotka, 1938) such as the bile acids, sex hormones, cardiac aglucones, toad poisons, digitalis sapogenins, etc. have enormously extended the physiological possibilities of the sterols.

Data on Tissue Lipids

Tables 22, 23, and 24 are given to show typical values for the lipids of various tissues.

Table 22. Lipids of Beef Tissues (Bloor, 1928).

Lipid Fraction	Weight (%)§	Fatty Acids* Solid (%)†	Liquid (%)†	Iodine Number T.F.A.	L.F.A.	4-Bond Acids (%)‡
		Liver				
Cephalin	1.50	18	40	119	179	19
Lecithin	1.56	20	46	108	160	21
Acetone-soluble	0.90	33	44	87	132	
Unsaponifiable	0.22					
		Kidney				
Cephalin	0.74	18	51	109	179	11
Lecithin	0.88	21	43	110	156	12
Acetone-soluble	0.90	34	46	82	136	
Unsaponifiable	0.19					
		Pancreas				
Cephalin	0.82	16	51	98	146	7
Lecithin	1.05	13	46	91	138	5
Acetone-soluble	3.90	31	44	62	109	
Unsaponifiable	1.29					
		Lung				
Cephalin	0.57	21	48	104	152	10
Lecithin	0.68	18	47	93	136	11
Acetone-soluble	0.76	27	50	76	114	
Unsaponifiable	0.25					

* Solid and liquid fatty acids are the weights of material as separated by the Twitchell procedure. Their sum is often considerably below that of the total fatty acids as determined directly, a difference which is believed to be due to unknown acids, the lead salts of which do not dissolve in hot alcohol, or to the presence of other alcohol-insoluble material, perhaps lignoceryl sphingosine (see page 209).

† In per cent of total fatty acids.

‡ In per cent of liquid fatty acids.

T.F.A. = Total fatty acids; L.F.A. = Liquid fatty acids. The acetone-soluble material is largely fat but may contain cholesterol esters, etc. The unsaponifiable has been found to be about half cholesterol.

§ In per cent of moist tissue.

Muscle

Normal variations. Lipid values for muscle vary widely with the type of muscle (heart, smooth, striated), the animal (warm-blooded, cold-blooded), and apparently with the degree of habitual activity of the

Table 23. Phospholipid* of Tissues (Javillier, Crémieu and Hinglais, 1928). (Grams per 100 Grams Fresh Material)

	Muscle	Heart	Brain	Liver	Kidney	Spleen
Horse	1.7	2.6	5.1	3 7	2.6	2.1
Guinea pig	1.5	2.9	4.7	2.9	2.8	2.7
Rat	1.1	3.0	6.1	2.9	2.9	2.0
Pigeon	2.4	2.8	4.7	2.9	3.3	
Frog	1.2			3.1	2.9	
Carp	1.1			2.5		
Dog-fish	1.1		4.7	1.7		

* Recalculated from lipid phosphorus (phospholipid = lipid phosphorus × 25). These values are considerably higher than those obtained by direct separation and determination of phospholipid. One reason for the difference is that lipid phosphorus always contains some non-lipid material (Le Breton, 1921). Another is that the factor is probably too high owing to the fact that the phospholipid often does not contain its full amount of fatty acid.

Table 24. Phospholipid Analyses of Normal Human Organs (Thannhauser and associates, 1939). (Mg. per 100 Mg. Dry Tissue)

	Total Phospholipid	Sphingo-myelin	Cephalin	Lecithin
Brain*	30.90	5.66	20.42	4.81
Lung	6.65	1.45	2.64	3.83
Spleen	8.56	0.86	4.16	3.54
Kidney	8.00	0.72	3.26	5.10
Liver	9.80	0.38	4.62	4.81
Heart	6.87	0.34	2.06	4.47

* Including both white and gray matter.

muscle. The heart muscle always has the highest phospholipid content of all the muscles in any particular animal, but may have a lower or higher phospholipid content than the heart muscle of another animal. Skeletal muscles show wide variations in value. Involuntary muscle always has a higher cholesterol content than voluntary muscle: 0.75 per cent of its dry weight as compared with 0.55 per cent for heart and 0.3 per cent for skeletal muscle, and since its phospholipid content is about the same as that of skeletal muscle its phospholipid-to-cholesterol ratio is always lower than that of skeletal muscle. The heart may have as high a cholesterol content as involuntary muscle, but since its phospholipid content is also high, the phospholipid-to-cholesterol ratio is generally the same as that of voluntary muscle. The ratio of phospholipid to cholesterol is about 10-16 to 1 in skeletal and heart muscle and 5 to 1 in smooth muscle, the latter difference in relation being due to the higher cholesterol content of smooth muscle. The phospholipid content of the muscles of very active animals has always been found higher than that of the corresponding muscles of sedentary animals of the same or different species (Bloor, 1936).

The neutral fat of muscles is stored fat of the same nature as that in other depots (Bloor, 1936). The fatty acids of the lipids of beef heart muscle were examined by Klenk and Ditt (1934), who found that the fatty acids of the neutral fat portion are not greatly different from those

of fat of other locations, containing only about 1 per cent of fatty acids with carbon chains longer than C_{18}. The phospholipid fatty acids, on the other hand, contained about 15 per cent of C_{20} and C_{22} acids with from two to four double bonds. Quagliariello (1921) studied the distribution of the fatty materials in muscle and concluded that the lipids are in the fibrils and not in the sarcoplasm.

Analyses of the lipids of muscle showing the amounts of the two constituents, phospholipid and cholesterol, and their relation to each other are given in the Tables 25, 26, and 27. Comments on the possible significance of these values as regards function of lipids in muscle are made later in the chapter (p. 250).

Table 25. Phospholipid and Cholesterol Content of Heart and Skeletal Muscle (Bloor, 1936).
(Per Cent of Dry Weight)

Animal	Heart (Ventricle)			Skeletal (Thigh)		
	Phospholipid	Cholesterol	P/C*	Phospholipid	Cholesterol	P/C*
Mammals						
Man	7	0.70	10			
Sea lion	5.6	0.40	14			
Kangaroo	6.72	0.32	21	2.38	0.17	14
Dog	8.54	0.61	14	8.00	0 32	25
Cat	5.72	0.44	13	2.47	0.19	13
Laboratory rabbit	9.12	0.57	16	1.70	0.17	10
Wild rabbit	7.65	0.45	17	3.75	0.25	15
Jack rabbit	7.22	0.38	19	8.75	0.35	25
Guinea pig	5 72	0.44	13	3.30	0.33	10
Gopher	9.6	0.80	12	7.50	0.30	25
Rat	7.95	0.53	15	3.50	0.25	14
Mouse				6 72	0.42	16
Average	7 65	0.51	15	4 59	0.27	17
Birds						
Wild duck	7 80	0.52	15	3.78	0.21	18
Hen	7.56	0.54	14	3.75	0.25	15
Pigeon	7.28	0 52	14	4.51	0.41	11
Sparrow	8 55	0.57	15	4.40	0.40	11
Owl	6.16	0.56	11	3.64	0.28	13
Average	7.56	0.54	14	4.34	0.31	14
Cold-blooded						
Turtle	6.40	0.80	8	3.50	0.35	10
Frog	4 20	0.70	6	3.80	0.20	19
Alligator	7.50	0 75	10	2.04	0.17	12
Grasshopper				5.40	0.18	30
Average	6.00	0.75	8	4.14	0.23	18
Special						
Turtle auricle	5.25	1.5	3.5			
Alligator auricle	7.20	1.8	4			
Bat pectoralis				7.98	0.57	14
New-born wild rabbit thigh				6.75	0.75	9

* Phospholipid/Cholesterol

In skeletal muscle, the phospholipids, lecithin and cephalin, are pres-

Table 26. Phospholipid and Cholesterol Content of Smooth Muscle (Bloor, 1936).
(Per Cent of Dry Weight)

Muscle	Phospholipid	Cholesterol	P/C*
Stomach (wild rabbit)	2.50	0.50	5
Intestine (cat)	3.24	0.81	4
Gizzard (hen)	2.00	0.50	4
" (pigeon)	2.52	0.63	4
" (owl)	3.50	1.00	3.5
" (sparrow)	2.88	0.72	4
Uterus (human)	3.50	1.00	3.5
" (laboratory rabbit)	4.40	1.10	4
" (dog)	3.00	1.00	3
Average of all smooth muscle	3.08	0.77	4

* Phospholipid/Cholesterol.

Table 27. Summary of Phospholipid and Cholesterol Content of Muscles (Bloor, 1936).
(Per Cent of Dry Weight)

Muscle	Phospholipid	Cholesterol	P/C*
Skeletal muscle	4.32	0 27	16
" " (vital)	4.42	0.34	13
Ventricle (warm-blooded animal)	7.70	0.55	14
" (cold " ")	6.16	0 77	8
Auricles of turtle and alligator	6.11	1.65	3.7
Smooth muscle (gastrointestinal tract)	2.80	0.70	4
" " (uterus)	3.68	1.05	3.5

* Phospholipid/Cholesterol.

ent in about equal amounts, lecithin being consistently somewhat higher (Bloor, 1927). In smooth muscle, especially that of the uterus, a definitely higher *lecithin/cephalin* ratio was found by MacLachlan (unpublished data from this laboratory). MacLachlan's determinations of lecithin made by calculation from the choline content gave values which agreed very well with those made by making use of the insolubility of cephalin in absolute alcohol. Sphingomyelin was found in small amounts in skeletal muscle and in somewhat larger proportions in heart muscle. The recoverable fatty acid content of muscle lecithin was generally close to the theoretical, but the recovery of cephalin fatty acids was always 8-10 per cent low, which emphasizes the fact that the exact nature of cephalin is not yet known.

The fatty acids of muscle phospholipid contain a relatively high proportion of liquid or unsaturated acids, the proportion being about one-fourth to one-third solid acids to two-thirds to three-fourths liquid over a wide range of muscles in a variety of animals. Snider (1936) found a considerable constancy in composition and nature of the phospholipid fatty acids in muscle, an average percentage of 73 for liquid acids and 27 for solid acids, as compared to a 60:40 ratio for liver (Snider and Bloor, 1933). The iodine absorption value of the liquid fatty acids was quite

constant at about 173, which is lower than that of the liquid fatty acids in liver. Sinclair (1935a) confirmed the constancy of the ratio of solid to liquid acids of phospholipids of muscle, liver, and kidneys of the rat and showed that differences in the degree of unsaturation were due to differences in the relative proportions of the various unsaturated acids, and not to differences in saturated acid content. The evidence indicates definitely that there is in muscle a high degree of selection of available fatty acids for the synthesis of phospholipid. The muscles seem to have a marked affinity for the more unsaturated fatty acids. Thus it has been shown by Sinclair (1932a) that when rats are transferred from a low-fat diet to one containing cod liver oil, the muscle phospholipids rapidly incorporate the unsaturated fatty acids, whereas the reverse process, the substitution of less saturated acids for the highly unsaturated acids is a slow process. The muscle phospholipids have a marked tendency to attain, and, having once attained, to maintain a high degree of unsaturation.

Other work of Sinclair, showing that the fatty acids of the phospholipids are to a considerable extent dependent on the nature of the fatty acids of the food, has given a new outlook on the metabolism of both fat and phospholipid in muscle. Using elaidic acid as his "labeled" fatty acid, he found that on a diet rich in elaidin, elaidic acid made up as much as one-third of the total fatty acids of skeletal muscles and liver. It replaced 25 to 30 per cent of the fatty acids and for the most part the fully saturated ones. (Since it is itself unsaturated, this fact indicates that melting point, rather than saturation, is the determining factor in the selection of the solid acids for the phospholipid.) The rate of entry of this "foreign" fatty acid into muscle phospholipid was slow (many days), whereas it was rapid into liver phospholipid (1 day); this fact led Sinclair to the belief that muscle phospholipid was of the structural or functional as opposed to the metabolic type, and that the slow entry of the new acid took place as the result of wear and tear and replacement in the phospholipid. It does not follow that the muscle may not make use of the metabolic type of phospholipid for energy production, but only that there is no great accumulation of it in the muscles. The great differences in content of phospholipid in different muscles might be due to phospholipid of either type.

Muscle lipids under various abnormal conditions. *Fasting.* In general, the finding of Terroine (1919) that except for the stored fat the lipids of muscle change very little in fasting is accepted, but it should be noted that not all workers are in agreement as to the effect of fasting on muscle phospholipid. Thus Cahn (1927) gives the following figures, which are expressed as grams per 100 grams of fresh muscle.

Lipid phosphorus of	Amount
Fresh muscle	0.044-0.061
Atrophied muscle	0.035-0.052
Muscle after fasting	0.034-0.038

Nutritional dystrophy. Nutritional dystrophy of muscles in rabbits (Morgulis, Wilder, Spencer, and Eppstein, 1938) produced a great increase in cholesterol, which these workers thought was probably the result of a synthesis. Only the skeletal muscle showed any increase of phospholipid along with the cholesterol. The heart and internal organs showed no change. In the brain, phospholipid increased, as did also cholesterol in larger proportion. Not all muscles were equally affected, the gastrocnemius being most and the abdominal muscles least affected. Cholesterol increased first, then the phospholipid, and finally the fat. With large increases of cholesterol there was an increase of cholesterol ester up to 12 to 27 per cent of the total cholesterol as compared with 4-8 per cent in normal muscles.

Injection of thyroidin. Thyroidin was found to increase the phospholipid content of muscle of rats up to nearly double (Pasternak and Page, 1935), and the increase was not due to interference with phospholipid metabolism since injected phospholipid was completely metabolized. There was found to be a 40 per cent increase in phospholipid in the whole animal and a corresponding rise in cholesterol. Thyroidin thus resulted in an absolute increase of phospholipid and cholesterol, which may be regarded as the result of stepping up the total metabolism resulting in increased use of fat.

Injection of thyroxin. Thyroxin (0.4-1 mg. daily for 10 days) was found by L. H. Schmidt (1935) to decrease liver and increase muscle phospholipid. In the liver there was an increase in non-phospholipid fatty acids (fat), in the muscles a decrease. The decrease of phosphocreatine in muscles after thyroid treatments observed by Bodansky (1935) may perhaps be related to the rise in phospholipid.

Brain and nervous system

Much work has been done on the lipids of the brain and nervous system, but most of it has to do with the fatty acids found in the various brain compounds and has been referred to in Chapter I. A few analyses of the lipids of brain and nerves are available. For the earliest investigation of the chemical composition of the brain, we are indebted to Thudichum (1901). The following analyses are taken from his work:

100 grams of moist substance contains in grams,

	Cholesterol	Cephalin	Lecithin
Gray substance	1.96	0.33	1.59
White substance	3.26	0.09	0.73

These values are lower than the more recent ones given below.

Analyses from this laboratory of beef brain (Bloor, 1928) gave the following average values in grams per 100 grams of moist whole brain:

Cephalin	2.36
Lecithin	2.23
Acetone-soluble, mostly cholesterol	1.88

The following comparative analyses of gray and white matter are given by Yasuda (1937):

	Water	Phospholipid % moist	Phospholipid % dry	I No.	Cholesterol % moist	Cholesterol % dry	Phospholipid / Cholesterol
White matter	68	12.5	39 3	66	4.3	13.4	2.9
Gray matter	83	4 4	25.2	92	1.1	6.3	4.0

Randall (1938a), in an attempt to relate function to chemical composition, has made extensive analyses of samples from various parts of the brain, including not only the lipids and lipid constituents but many inorganic constituents. Some of his findings are as follows: The water content is higher in gray matter than in white, and lipids, including lipid phosphorus and nitrogen, are higher in the white substance. The iodine number of the phospholipid fatty acids is higher in the gray substance than in the white (*e.g.*, frontal white matter—84, frontal gray matter—129). The various gray and white areas were not distinguishable by their composition.

Neutral fat in notable amounts is generally not found in adult brain. Tuthill (1938) determined the fat content of the brains of forty-six infants from birth to two years and found fat around the blood vessels and glia cells of the centrum ovale, corpus callosum, and white substance of the lower part of the gyri in all infants less than four months of age. The fat decreased with the formation of the myelin and disappeared at four months when myelinization is apparently complete. Perivascular fat occurs in the large subcortical vessels from the first month to the end of two years of life.

The cerebrosides of the brain of various animals were made the subject of study by Lanfranchi (1938). He found that cerebrosides were lacking in the brain of the octopus. In teleost fishes, they constituted 3.39 per cent of the dry weight; in cartilaginous fish, 3.69; in the toad, 3.1; turtle, 6.09; fowl, 6.02; and mammal, 10.23; they therefore increase with phylogenetic evolution. In hatching chickens the amount present on the fifteenth day was 3 times that on the tenth day, and, on the twentieth day, 11 times as great.

The fatty acids found in the brain lipids are notable because of their high molecular weight and high degree of unsaturation. As to their exact nature, there is a difference of opinion. Levene and Rolf (1922) found

arachidonic acid to be the most important of these. Klenk (1929) reported that C_{24} acids occur; he was impressed by the frequency of occurrence of multiples of six carbon atoms in the fatty acids of brain, for example, C_{18} and C_{24} fatty acids, as well as C_{18} in sphingosine, and C_6 in galactose, which suggested to him a common origin in the hexose sugars. Brown and Ault (1930) studied the unsaturated fatty acids of animal brains by the preparation of their bromine absorption compounds. The ether-insoluble bromides from hog brains showed that arachidonic acid was probably the main constituent, although the melting point of the bromide was too high. In sheep and beef brains, they found evidence of a more unsaturated acid, tetracosapentenoic (C_{24}). The molecular weights of the brain fatty acids were high, mostly well over 300. In later work, Brown (1932) found only C_{22} acids and no arachidonic acid (C_{20}) or C_{24} acids, which is a reversal of his earlier conclusion. Klenk and von Schoenebeck (1931) found that the highly unsaturated C_{22} acid in brain cephalin occurred also in liver phospholipid.

In the ether-insoluble lecithin of brain, Merz (1931) found mainly saturated acids, a large proportion of which was palmitic with very little stearic, and only 21 per cent of unsaturated acid, which he found to be oleic; 58 per cent of the glycerophosphoric acid was of the β form. Three sphingomyelins were found containing, respectively, stearic, lignoceric, and nervonic acids. Page and Rudy (1932) found in a cephalin from human brain: stearic acid, 30 per cent; a C_{22} unsaturated acid, 22 per cent; unsaturated acids of the C_{18} series, 22 per cent; and possibly a C_{20} acid. Yokoyama and Suzuki (1932) found oleic, arachidonic and palmitic acids in the α-lecithins of human brains and the same plus linoleic acid in the β-lecithins. Blix (1933) found a sulfur-containing substance in brain and considered it to be probably the potassium salt of a cerebron sulfuric acid ester. It makes up a quarter to a fifth of the total brain cerebroside and is apparently the only sulfur-containing lipid of the brain.

Workers are agreed that it is very difficult to change the composition of the brain lipids by changes in the diet. Thus, Stoesser, Petri and McQuarrie (1935) found that in spite of wide differences in diet there was little variation in composition of rats' brains. However, cholesterol increased significantly with age. McConnell and Sinclair (1937b) found that, although it was very difficult to change appreciably the composition of the brain of the adult rat by diet, yet characteristic fatty acids of the food (in this case elaidic acid) could be introduced into the brain phospholipid of the young rat through the mother by way of the placenta and the milk. Even so, however, the elaidic acid content of the brain phospholipid was only about one-fourth that of the liver and the muscle of the same animals, indicating a much greater degree of selection in the

case of the brain than of the other tissues. Changus, Chaikoff and Ruben (1938), using radioactive phosphorus, showed that the change in brain phosphorus was the slowest of all the tissues; but the increase was progressive for 200 hours after a single dose and it decreased at a correspondingly slow rate up until 4 weeks after. The uptake was much faster in the brains of young animals than in those of older ones.

Comparisons of the lipid and nitrogen contents of various parts of the nervous system of dogs were made by Palladin, Rashba and Helman (1935a). In the following table, the parts are listed in order of decreasing content:

Cholesterol and Unsaturated Phosphatide	Total Nitrogen	Creatine	Creatine N / Total N	
Spinal cord (gray)	Cerebral cortex	Cortex cerebelli	Cortex cerebelli	4.20
Nucleus caudatus	Cortex cerebelli	Nucleus caudatus	Nucleus caudatus	3.94
Cerebral cortex	Nucleus caudatus	Cerebral cortex	Spinal cord	3 42
Cortex cerebelli	Spinal cord	Spinal cord	Cerebral cortex	2.66

The amounts of saturated phosphatide and cerebrosides in the spinal cord were less than in the other parts; the spinal cord contained more dry residue than the other parts. The total cholesterol in the spinal cord of cattle is given by Pfeiffer (1931b) as 0.61 per cent, of which 38 per cent was combined as esters of oleic acid.

Palladin, Rashba and Helman (1935b) also investigated the vegetative nervous system of cows. The table below gives the contents in decreasing order:

Unsat'd Phosphatide / Acid-sol. Phosphorus	Saturated Phosphatide	Total Phosphorus / Dry Residue	Total Phosphorus	Dry Residue Values
g. coeliacus	g. coeliacus (0.439)	g. coeliacus	1.777	24.4
g. of symp. trunk	g. nodosum n. vagi	g. of symp. trunk	0.939	21.1
g. nodosum n. vagi	g. of symp. trunk	g. nodosum n. vagi	0.836	20.5

The ganglion nodosum n. vagi has a higher content of cholesterol than have the ganglion coeliacus and the ganglion of the sympathetic trunk. The total nitrogen is equally distributed. The values of residual nitrogen exhibit considerable individual variations. The proportion of creatine nitrogen is very low, especially in ganglion nodosum n. vagi. The ganglia of the sympathetic and parasympathetic nervous systems differ in biological functions and in chemical composition.

Many attempts have been made to connect function with composition of the brain, to determine the localization of the fatty substances in different areas, and to examine changes in composition during development and degeneration. In examining these attempts to correlate composition and structure, the fact must be kept in mind that by far the larger portion of the brain and nervous system consists of conducting material, which is functionally relatively passive.

Gorodisskay (1925) made a study of the distribution of the various lipids in different areas of the brain. She found that the percentage of cholesterol differed considerably in the different areas; the saturated phospholipids were next in variability, and the unsaturated phospholipids least variable. Calculated with reference to the total nitrogen, the lowest cholesterol values were found in the sphere of the higher psychic function and the highest values in the motor areas. The two sides of the brain had different compositions. In general, the more important and more active the portion of the nervous system, the less lipid and more protein it contained. The highest lipid content was found in the peripheral nerves, a lower content in the spinal cord, and still lower in the brain. In the gray matter, the lipids constituted one-third, and in the white matter two-thirds, of the dry substance. The gray matter of the cerebrum was poorer in lipids and higher in protein than that of the subcortical ganglia. The highest lipid content was found in the motor centers, the lowest in the association centers. Especially low values were found for cholesterol, cerebrosides, and saturated phospholipids in the latter centers.

Gorodisskay also studied the effects of age and found that up to 45 years there was no definite relation of age to composition, but after that the unsaturated phospholipids and proteins diminished, and cholesterol increased; the saturated phospholipid and cerebrosides did not change. The changes with age were most marked in the association centers.

Backlin (1930) contributed a study of the changes in the lipids of rabbit brain during development. He found, as have practically all workers, that at birth the cerebrosides were lacking in the brain and their appearance and increase paralleled that of the myelin sheath of the nerves. During extra-uterine development the dry substance increased, total lipid increased in terms of dry matter, and all lipid fractions increased in terms of moist weight, the change being least for the phospholipids. The most notable change was in the cerebrosides, which were almost zero at birth but increased greatly after birth. The unsaturated phospholipids probably did not increase in percentage, but the saturated phospholipids did. The saturated phospholipids, cholesterol, and cerebrosides increased much more than the unsaturated phospholipids. McConnell and Sinclair (1937b) gave the following figures for phospholipids during growth of rats in terms of lecithin and cephalin fatty acids of moist brain: new-born (av.) 1.20, three weeks 2.25, three months 3.0, which are in good agreement with the earlier ones of Koch and Koch (1913). Lang (1937) found that the phospholipids of the brains of young rats increased from 17.3 per cent of dry weight at one day to 25.1 per cent in the grown animal. Cholesterol increased from 3.27 to 7.62 per cent.

May (1930) made a study of degeneration in brain and nerve tissue on autopsy material and found that degenerative changes consisted largely of a lowering of the phospholipid content. In his first paper, he studied the percentages of sulfur and phosphorus in the two hemispheres of the guinea-pig brain and found that they were the same. In a second paper, he produced traumatic encephalitis of one hemisphere and determined total nitrogen, water content, sulfur, and phosphorus during the first eight days of disintegration, and then twenty-nine days after, using the uninjured hemisphere as control. He found that the main changes produced by the degeneration were in the phospholipid, which diminished greatly. When a nerve was cut and allowed to degenerate, the main changes found were degradation of the phospholipids and nucleoproteins. Laurie (1933) compared the lipid content of the gray and white matter in normal and demented persons and could find a definite difference only in the unsaponifiable fraction of the white substance, which was markedly higher in the demented persons than in the normals. The water content in the white substance was lower in the demented than in the normal.

Falk (1908) gives the following analyses of nerves, expressed in per cent of lipid:

	Total Extract (% tissue)	Cholesterol	Cephalin	Lecithin	Cerebrosides
Non-medullated	11 5	47.0	23.7	9.8	6 0
Medullated	46.6	25.0	12.4	2.9	18.2

In the non-medullated fibers, the phospholipids were more largely represented; in medullated, the cerebrosides were characteristic. Cephalin was the main phospholipid in both.

A study of the lipid content of nerves has been made by Randall (1938b). For normal human nerves, the average composition in percentage of dry weight was as follows. The number of samples analyzed is given in parentheses.

	Phospholipid	Cholesterol	Cerebroside	Fat (moist wt.)	Water
Femoral (23)	13.36	4.37	5.36	9.05	65.0
Sciatic (3)	13.58	4.59	4.52	9.03	68.8
Post tibial (12)	13.13	4.37	4.24	8.04	68.3

Nerves from arteriosclerotic subjects, both diabetic and otherwise, show great losses (50-60 per cent) in phospholipid, cholesterol, and cerebroside with corresponding gains in fat and water.

Liver

The liver takes an important part in the metabolism of the lipids, and for this reason the changes in the content and composition of the

fatty substances in the liver have special significance. Much of the work bearing on this subject will be discussed under "Metabolism" and so need be mentioned only briefly here. The liver may serve as an emergency storage organ for fat and cholesterol esters, although these are ordinarily not stored there in considerable amounts. Since the bile contains cholesterol, the liver is probably important in the excretion of this substance. In fasting and in various types of poisoning, it is well known that the stored fat of the depots and tissues is mobilized to the liver. During fat digestion, the absorbed fat also goes there, and it has been shown by the use of labeled fat that the absorbed fat is represented to a considerable extent in the liver phospholipids. This phospholipid disappears in a short time and may then be demonstrated in the tissues. Phosphorylation of the absorbed fats has been shown to take place in the liver as well as in the intestine (Perlman and Chaikoff, 1939). Under special conditions—in depancreatized animals, in animals on a high-fat diet, and in animals fed cholesterol, cystine, etc.—the amount of fat accumulated in the liver may reach high levels. Along with it, there are also cholesterol esters of the fatty acids. This abnormal accumulation may be prevented and abolished by choline and similar substances; and it is a short step to the assumptions that the reason for the accumulation is failure to form phospholipid due to choline deficiency and that when choline is supplied, not only does the fat move out as phospholipid but the cholesterol ester accumulation also diminishes.

Representatives of most of the known groups of lipids are found in the liver, and in the normal postabsorptive state the amounts present are probably those characteristic of the liver as a tissue. The lipid present in largest amount is phospholipid; neutral fat is generally present in smaller amounts (see Table 28). Of the phospholipids, the most important quantitatively in beef liver are lecithin and cephalin, which are present in nearly equal amounts (Bloor, 1928). A polysphingomyelin from liver has been described by Fränkel, Bielschowsky and Thannhauser (1933), which contains equal parts of lignoceric, palmitic and stearic acids. It has a nitrogen-to-phosphorus ratio of 2:1. Enzymes from different sources were tried on this compound by Rossi (1935), and although they were all effective on glycerophosphate, they varied in their activity on this sphingomyelin.

The "unsaponifiable matter" of liver consists of sterols, mostly cholesterol; but in some cases, various hydrocarbons and higher alcohols, also residues from sphingomyelin, *e.g.*, lignoceryl sphingosine, are present. The hydrocarbons and higher alcohols are found mainly in fish livers. Fränkel and Löhr (1933) found in mammalian liver, in addition to cholesterol, a yellow sterol-like substance not precipitable by digitonin, but

reacting with the Liebermann-Burchard and Salkowski reagents, and also sphingosine and lignoceryl sphingosine. Thannhauser and Fränkel (1931) described the preparation of lignoceryl sphingosine from hog liver. It had a melting point of 90-90.5°C. and after hydrolysis with 10 per cent sulfuric acid yielded lignoceric acid and sphingosine sulfate. Freytag and Smith (1933) reported that sterols constituted 64 per cent of the unsaponifiable portion of beef liver and consisted mostly of cholesterol, dihydrocholesterol and ergosterol being present in small amounts. Lignoceryl sphingosine was identified. Vitamin A was found in relatively large amounts, vitamin E in traces, and an antioxidant in traces.

Analyses of the lipids of beef liver made by Theis (1928) and Bloor (1928) are compared in Table 28.

Table 28. Comparison of Data of Theis (1928) and Bloor (1928) on Normal Beef Liver Lipids.

	Theis	Bloor
Total lipid, weight (%)	4.6	4.2
Phospholipid { weight (%)	2.53	3.08
Phospholipid { total lipid (%)	55.0	73.0
Acetone-soluble (fat) { weight (%)	2.07	1.12
Acetone-soluble (fat) { total lipid (%)	45.0	27.0
Phospholipid fraction		
Iodine number of total phospholipid	100	82
Iodine number of mixed fatty acids	141	114
Iodine number of liquid fatty acids	246	170
Liquid acids, per cent of mixed acids	57.0	43.0
4-Bond acids, per cent of liquid acids	36.0	20.0
Fat fraction (acetone-soluble)		
Fatty acids (% content)	70.0	70.0

Tsujimoto and Kimura (1928) gave the following values for the fat of whale liver: total fatty material 6 per cent, of which 69.3 per cent was fatty acid and 16.1 per cent unsaponifiable. The fatty acids had a melting point of 32-34°C., a neutralization number of 194, and an iodine number of 141. They formed ether-insoluble bromine addition products, with a bromine content of 70.5 per cent. The liquid acids, 75.4 per cent of the whole, had an iodine number of 176 and a neutralization number of 189. The solid acids constituting the remaining 25 per cent had a melting point of 52-53°C. The unsaponifiable substance contained 48 per cent of cholesterol, and an unsaturated hydrocarbon, $C_{35}H_{60}$.

In elasmobranch livers, Tsujimoto (1932) found pristane ($C_{18}H_{38}$), squalene ($C_{30}H_{50}$) (also called spinacene), chemyl alcohol ($C_{19}H_{40}O_3$), batyl alcohol ($C_{21}H_{44}O_3$), selachyl alcohol ($C_{21}H_{42}O_3$) and selacholeic acid ($C_{24}H_{46}O_2$), m.p. 42.5-43°C., a Δ^{14} tetracosenoic acid.

In the liver oil of a small shark (*Emopterus spinax*), Klenk (1933a) found more than 50 per cent of unsaponifiable matter, including an abundance of squalene ($C_{30}H_{50}$). In the liver fats, he found C_{16}, C_{18}, C_{20}, C_{22}, and C_{24} acids and in the liver phospholipids, along with a small amount of saturated acid, mostly unsaturated acids of the C_{18} and C_{22} groups.

Guha, Hilditch and Lovern (1930) found in fish-liver oils that when squalene (I No. 370) was high in percentage, the fatty acids present had a low iodine number. In skate-liver oil there was no squalene but much of unsaturated C_{20} and C_{22} acids. Lovern (1930) found in the liver of the thrasher shark that the oils had an iodine number of 176 with unsaponifiable substance 1.83 per cent, of which cholesterol constituted 22 per cent. The solid acids were myristic, palmitic, and stearic. Among the liquid acids were C_{14}, C_{16}, C_{18}, C_{20}, and C_{22} acids.

Klenk (1933b) examined the fatty acids of frog liver phospholipids and found, in comparison with those of higher vertebrates, a low content of stearic acid and a relatively high content of highly unsaturated acids of the C_{20} and C_{22} groups. In the fat, there was found palmitoleic, linoleic, linolenic, arachidonic, and clupanodonic acids. Frog fat, in general, resembles the fat of fresh-water fishes, and occupies a position intermediate between the fat of land mammals and that of marine fishes.

The pioneer work in the field of liver fatty acids was done by Hartley (1907, 1909), who reported the presence of arachidonic and other high-molecular unsaturated acids and an oleic acid different from ordinary oleic acid [double bond in the 12-13 position; later investigators (Channon, Irving and Smith, 1934) have not been able to confirm the presence of this acid]. The mean molecular weight of the fatty acids was found by titration to be 308-312, indicating the presence of acids of greater than C_{18} and foreshadowing the findings of Klenk and others later. Brown (1928) found that arachidonic was the only highly unsaturated acid in liver, and that it was present to the extent of 2 to 7 per cent of the total fatty acids. Wesson (1925) found that feeding cod liver oil to rats resulted in increasing the arachidonic acid in the tissues up to four-fold. Fasting increased arachidonic acid in the store. Klenk and von Schoenebeck (1932) found also some C_{22} acid with an average of 4.1 double bonds.

Irving and Smith (1935) list the following fatty acids of pig's liver: *n*-decenoic and lauric 0.4, myristic 0.7, palmitic 14.0, stearic 18.8, arachidic 1.7, palmitoleic 1.5, oleic 28, linoleic 5, linolenic 0, C_{20} 20, and C_{22} 7.5 in per cent of original mixture.

The neutral fat of beef liver was found by Klenk and von Schoenebeck (1932) to contain the amounts of fatty acids shown in Table 29:

Table 29. The Fatty Acid Content of the Neutral Fat of Beef Liver (Klenk and von Schoenebeck, 1932).
(Per Cent of Liver Fat)

	Solid Acids	Liquid Acids	
			I No.
C_{14}	trace	trace	
C_{16}	25	9	?
C_{18}	20	37	114
C_{20}	trace	8	200
C_{22}	trace	1	270

The depot fat of beef contained nothing higher than C_{18} and only about 6 per cent of C_{14}, of which one part is saturated and five parts unsaturated (Banks and Hilditch, 1931). The highly unsaturated acids of the liver were C_{20} and C_{24}, which are not represented in the depot fat and therefore could not have originated by simple desaturation of depot fat, according to Leathes' conception; the highly unsaturated acids of plants are mostly C_{18} acids. The C_{18} acids of the liver, which may have originated in the depots, have only a low degree of unsaturation such as might be found in a mixture of oleic and linoleic acids.

Hilditch and Shorland (1937) found that the non-phospholipid fatty acids of the liver of New Zealand farm animals were like those of the depot fat except that they contain 5 to 10 per cent (mol.) of hexadecenoic and 5 to 15 per cent (mol.) of C_{20} and C_{22} highly unsaturated acids, a much higher content than the depot fats. The phospholipid fatty acids are characterized by an increased proportion of stearic, and C_{20} and C_{22} unsaturated acids with less hexadecenoic, as compared with the corresponding liver glycerides. The liver phospholipids tend definitely to contain acids of higher molecular weight than the neutral fat.

An excellent study of the fatty acids of liver phospholipids (of beef) is that of Klenk and von Schoenebeck (1932) made by distillation of the methyl esters. The results from 1800 grams of phospholipids are given in Table 30.

Table 30. The Fatty Acid Content of the Phospholipids of Beef Liver (Klenk and von Schoenebeck, 1932).
(Grams in 1800 Grams of Liver Phospholipid)

Methyl Ester	Saturated Fatty Acids	Weakly Unsaturated Fatty Acids: Amount	Weakly Unsaturated Fatty Acids: I No.	Highly Unsaturated Fatty Acids: Amount	Highly Unsaturated Fatty Acids: I No.
C_{14}				trace	
C_{16}	75	12 (palm.)		16	?
C_{18}	163	81	115	83	151
C_{20}		10	207	100	253
C_{22}		10	207	53	302
C_{24}	2				

The values for the C_{20} and C_{22} acids are too low since the acids polymerize on distillation and are lost by oxidation. The C_{24} solid acid is probably lignoceric from sphingomyelin. The C_{18} acids are oleic and linoleic, the C_{20} arachidonic, the C_{22} clupanodonic. In general, the fatty acids of the liver phospholipids are the same as those of the brain, but in brain C_{22} acids predominate, while in the liver the C_{20} acids predominate. In brain, only traces of C_{18} acids more highly unsaturated than oleic occur; in liver, linoleic is abundant. It must be kept in mind, however, that the liver phospholipids respond quickly to absorbed fat and to a very

considerable extent contain fatty acids which have recently been absorbed from the intestine (Sinclair, 1930). On the other hand, there are undoubtedly some fatty acids of the liver phospholipids which are independent of the food fat. These would probably be the ones not found in food fat, for example, palmitoleic and the C_{20}, C_{22}, and C_{24} acids.

In liver lecithin, Levene and Ingvaldsen (1920) found stearic and linoleic acids, but no oleic. Levene and Simms (1921) found both palmitic and stearic acids, and among the unsaturated acids, arachidonic and one other.

During aseptic autolysis of the liver, the phospholipid diminishes because of liberation of the fatty acids by hydrolysis.

Effect of various factors. The fat of the liver, according to present views, represents mostly food or depot fat temporarily stored there on the way to use in metabolism. Such loosely stored fat might be expected to be readily moved either in or out of the liver under varying conditions. The mobility of this fat store is shown by the work of Ohlsson and Blix (1934), who found that the neutral fat of rat liver undergoes diurnal variations which are independent of food intake, accumulating during the early hours of the day and diminishing during the afternoon. The high-fat phase coincides with the low-glycogen phase, and vice versa. No cyclic changes were observed in the phospholipid or water content. It has also been shown that low barometric pressure or low oxygen tension causes a mobilization of fat to the liver when there is available fat in the depots (Monasterio, 1930; Loewy and Cronheim, 1933).

Flock, Bollman and Mann (1936) found the amount of lipid phosphorus in the liver of the dog less subject to change as the result of diet than the other forms of phosphorus.

In laying hens, the neutral fat of the liver was much higher than in the male or the immature female (Lorenz, Chaikoff and Entenman, 1938). In the blood, the neutral fat was much higher in the laying hen, but there was also a significant increase in phospholipid and cholesterol esters which did not occur in the liver.

Imrie and Graham (1920) made observations on the fat of the livers of embryonic guinea pigs throughout gestation. Until a weight of 35-40 grams was reached, the fat content approximated that of the mother (2-3 per cent). After this period, there was a progressive increase until birth, when the content reached 16-18 per cent of the moist tissue, the iodine number being high, but lower than that of the fat in the maternal liver. This store of fat was rapidly utilized by the animal in the first 48-72 hours after birth.

Torrisi (1934) found that the lipid phosphorus of the liver in the newborn dog was only slightly less than in the mother.

Lang (1937) found in growing rats from one day old to maturity that the liver phospholipid was approximately constant, but that the cholesterol rose to a sharp maximum about the fifteenth day and then fell, because of utilization in the rapid growth period beginning at twenty days.

The effect of the hormones from the organs of internal secretion has been examined by several investigators. Coope and Mottram (1914) found increased fat in the liver in late pregnancy, along with increased pituitary action. Coope and Chamberlain (1925) found that injection of pituitrin into rabbits doubled the fat of the liver, an effect which disappeared in 30 hours. In Fröhlich's syndrome (lack of pituitary secretion) the depot fat increased gently because of lack of stimulation to mobilize. Injection of extracts of the anterior pituitary into rats caused an increase of 50 per cent in the fat content of their livers (Anselmino, Hoffman and Rhoden, 1936). MacKay and Barnes (1938b) found that the fatty livers produced by injection of pituitary extract were not influenced either by choline or pancreas extract, and that the resulting ketonuria was slightly reduced by choline and not affected by pancreas extract.

Thyroxin injected into normal rabbits caused the phospholipid to diminish while the other lipids increased. The reverse took place in the skeletal muscles, while in blood both increased (C. F. Schmidt, 1935). Extirpation of the thyroid caused a diminution of the phospholipid fatty acids of liver, the other lipid fatty acids remaining normal or increasing slightly. Cholesterol increased greatly (Artom, 1923a).

Artom and Marziani (1924) compared analytically the lipid content of the livers of rabbits with and without ovariectomy, obtaining the following values, expressed in grams per 100 grams of moist tissue. There was an increase in water in the ovariectomized animals.

Animal	Phospholipid Fatty Acid	Other Fatty Acid	Total Fatty Acid	Cholesterol	Other Unsaponifiable
Normal	2 8	0.43	3.20	0.17	0.19
Ovariectomized	2.2	0.92	3.07	0.14	0.17

MacLachlan (1936a) studied the effect of insulin in large doses and infection by Aspergillus and Sporothrix on the liver and also the effect of liver damage by chloroform. Beyond causing the appearance of fat globules, there was very little effect either on the percentage or chemical nature of the liver lipids in the rat. MacLachlan and Hodge (1939), after cocaine feeding in mice, found neutral fat and cholesterol greatly increased, and phospholipid unchanged.

Infections, when they produced any effect, did so on the neutral fat alone. Phospholipid and cholesterol were unaffected, even when the fat accumulation was large or when it disappeared. Diphtheria caused a

disappearance of fat; paratyphoid had no effect; other infections generally produced fatty livers (Scheff and Horner, 1932).

Immunization (Wadsworth, Hyman and Nichols, 1935) of horses produced in their livers a lowering of phospholipid and an increase of free cholesterol and neutral fat, together with greater variations than in normal or resting horses.

That the mobilization of fat to the liver is under nervous control is indicated by the fact that the fatty liver produced by phlorizin poisoning can be prevented by section of the spinal cord at the level of the sixth thoracic vertebra (Wertheimer, 1931).

The most important of the various factors which affect the lipid composition of the liver are those conditions which produce fatty livers. The most pronounced change is that in the neutral fat, which increases from the normal 2 or 3 per cent up to 50 or 60 per cent in extreme cases. Along with the fat, especially when there is much cholesterol in the food, there is an accumulation of cholesterol, mainly in the form of cholesterol esters. In these fatty livers, the phospholipid remains normal in amount. The accumulation is regarded as representing a slowing up or blocking of the normal processes of fat metabolism which take place in the liver, although it is not certainly known just what these processes are. Removal of or interference with the normal activity of the pancreas, including its external secretion, is one of the important factors producing fatty livers. Artom (1923b) noted that there was little change in the percentage of phospholipid fatty acids, although the fatty acids of the neutral fat might be enormously increased in these fatty livers. Cholesterol was increased, but not greatly. MacLean and Best (1934) found that depancreatized dogs, even though given adequate treatment with insulin, eventually developed fatty livers. Choline prevented the accumulation. Normal rats fed a high-fat diet developed fatty livers, and again choline was found to accelerate the rate of removal of the extra fat under a variety of dietary conditions, which led to the conclusion that choline or similar substances may prove to be a significant and perhaps essential dietary factor (Best and Huntsman, 1935). It has also been reported that hypophysectomy did not prevent the fatty livers of pancreatectomy (Chaikoff, Gibbs, Holtom and Reichert, 1936).

Feeding cholesterol to rats causes an accumulation of cholesterol esters in the liver (up to 4.35 per cent from the normal 0.036 per cent) along with the fat, and choline is effective in causing the removal of the cholesterol esters as well as the fat (Best, Channon and Ridout, 1934). Choline is notably more effective against fat than against cholesterol esters, indicating that choline acts primarily on the glyceride fraction of the liver

lipids (Best and Ridout, 1935). Chanutin and Ludewig (1933), on feeding cholesterol to rats, found that the liver was the only organ showing great changes. Fat, cholesterol, and cholesterol esters progressively increased, while phospholipid remained normal. Okey (1923) fed cholesterol to rats at the rate of 1 mg. per gram of rat and found not only a great increase of neutral fat in the livers over the controls, but a phenomenal increase of cholesterol esters. Thus, on a 15 per cent fat diet, the controls showed a liver fat of 12 per cent and cholesterol ester of 0.19, as compared with 23 per cent fat and 7.25 per cent cholesterol esters in the experimental animals.

A high-fat diet will produce a fatty liver in most animals (Flock, Bollman, Hester and Mann, 1937). Feeding of lecithin or choline generally prevents the accumulation. Betaine has a similar effect (Best and Huntsman, 1932; Best, Hershey and Huntsman, 1932). Fats vary in their ability to produce fatty livers (Channon and Wilkinson, 1936). Butter is most effective, and, in general, the fats seem to vary inversely with their degree of unsaturation. Phospholipid percentage falls and cholesterol ester percentage rises with increased fat deposition. The deposited fat is not unchanged food fat but tends to approach a constant composition. Small amounts of cystine (0.05-1 per cent) added to a high-fat, low-casein diet produced a 50 per cent fat increase and 25 per cent increase of liver weight (Beeston and Channon, 1936). Tucker and Eckstein (1937), under these conditions, found that cystine gave a 57 per cent increase of liver fat while methionine gave a 41 per cent decrease. Casein has been found (Beeston, Channon, Loach and Wilkinson, 1936) moderately effective in preventing fatty livers (1 gram casein equivalent to 7-8 mg. choline). Gelatin has no lipotropic action, but edestin has. Egg yolk, which contains both cholesterol and phospholipid, gave interesting results in the work of Okey, Yokela and Knock (1934). In rats raised from weaning on dried egg yolk powder for 60 days with good growth, the livers of the control males had 7.5 per cent total fatty acids as compared with 9.3 in the experimental animals. The control females had 7.4 per cent, as compared with 11.7 per cent in the experimental females. Total cholesterol, males, was 0.48 per cent for the controls, and 2.58 per cent for experimental animals. In the females, the values were 0.30 and 3.86 per cent, respectively. Free cholesterol was 0.3 per cent in both. Lecithin in this case, although present in large amounts, did not prevent accumulation of large amounts of cholesterol esters. Okey and Yokela (1936), in later work, fed egg yolk protein and cholesterol with hydrogenated cottonseed oil and no lecithin for 120 days, obtaining still greater accumulations of fat and cholesterol esters. These accumulations, as before, were greater

in the females than in the males. Okey, Gillum and Yokela (1934) found that in rats on a diet containing 1 per cent of cholesterol for 60 days, the females stored less cholesterol in their livers than the males, the difference being in the form of esters, the inert form of cholesterol. It was thought that females had more need for cholesterol, possibly as sex hormones. Female rats form acetone bodies from fat in larger amounts than males (Deuel, Grunewald and Cutler, 1933), which may result in less fatty acid being available for cholesterol ester formation.

Liver feeding has been found by Blatherwick and associates (1933) to develop fatty livers with much fat and cholesterol esters. Cooked eggs increased the cholesterol up to 5.5 per cent and to a less extent the fat of livers. Lecithin had no opposing effect. The active substance was soluble in both water and 95 per cent alcohol.

From dog pancreas by alcohol extraction, Dragstedt, von Prohaska and Harms (1936) prepared a hormone (lipocaic) which, on the basis of histological evidence, they claimed was fifteen times as effective as choline in curing fatty livers. A number of workers repeated their investigations (MacKay and Barnes, 1938a; Aylward and Holt, 1937; Best and Ridout, 1938) and found that the effects produced could be adequately explained by the choline and other active substances in the extract. Ralli, Rubin and Present (1938) found that ligation of the pancreatic duct in dogs produced the same fatty livers as in depancreatized dogs, and concluded that there could be no fat metabolism hormone in the pancreas. Lipocaic as a specific hormone apparently needs further support.

In view of the fact that choline is an important constituent of lecithin, and since lecithin appears to be a primary step in fat metabolism, the conception arises that fatty livers are the result of inability to form lecithin fast enough to take care of a large amount of fat, which in turn is caused by deficiency of choline. To obtain evidence as to whether the preventive action of choline was exercised through lecithin formation, Channon and associates (1937b) used a substance homologous to choline,

triethylcholine, $N \begin{cases} CH_2.CH_2OH \\ \equiv (C_2H_5)_3 \\ OH \end{cases}$, instead of the ordinary trimethylcholine.

The effects of the two substances are the same, and the ethyl compound could be separated from the methyl derivative by reason of the lower solubility of its gold chloride. No evidence of the ethyl compound could be found in the phospholipids of livers made fatty in the usual way. The results fail to support the lecithin hypothesis, although they do not exclude it.

Channon and associates (1937a), in addition to the choline homolog

just mentioned, tried the effect of homocholine, $N \equiv (CH_3)_3 \begin{smallmatrix} \diagup CH_2OH.CH_2.CH_3 \\ \diagdown OH \end{smallmatrix}$, and found its effect on fatty livers greater than that of choline, as regards both neutral fat and cholesterol esters. Tripropylcholine had no effect; triethylcholine was less effective than ordinary choline. Welch (1936) contributed the interesting information that the arsenic homolog of choline, $HOC_2H_4.As(CH_3)_3.OH$, appears in traces in the phospholipids of brain and liver after being fed to rats. Betaine, $N \equiv (CH_3)_3 \begin{smallmatrix} \diagup CH_2.COOH \\ \diagdown OH \end{smallmatrix}$, had been found effective by Best and Huntsman (1932). Whether these substances are effective as such or because they yield choline has not been determined. The fact that they are mostly less effective than choline would favor the latter theory. Direct evidence that choline is the limiting factor in the production of fatty livers is available in the work of Perlman and Chaikoff (1939), who fed radioactive phosphorus along with choline. It was shown that choline increased phospholipid metabolism in the liver with increased formation and more rapid removal of phospholipid. With a single dose of choline, increased phospholipid metabolism appeared in approximately one hour, and its effect had disappeared by 10-12 hours thereafter. The increase in phospholipid formation was proportional to the dose of choline. Choline then may be either a catalyst or a material directly essential in phospholipid formation.

Other studies on various factors concerned with fatty livers have been made by Chaikoff and his associates. Chaikoff and Kaplan (1937) examined the distribution of fat in different lobes of the livers of fifteen depancreatized dogs. As fat accumulated, it was not evenly distributed in the lobes of the whole liver. Fatty (19.7 per cent) and normal (4.3 per cent) contents may exist in the same lobe (av. 10.6 per cent fat). Whole lobes were a better index than parts of the same lobe. Lipids in the livers of depancreatized animals may fluctuate from 3 to 66 per cent, glycogen from 0.05 to 10.5, water from 28 to 72 per cent, nitrogen from 0.9 to 3.22 per cent. Water did not accompany either glycogen or fat storage, but was parallel to the protein and decreased linearly as the sum of glycogen and fat increased. Fat storage did not interfere with glycogen storage. On the other hand, Ohlsson and Blix (1934) found that glycogen and fat storage in normal rats varied oppositely during the day. When fat was high, glycogen was low. In depancreatized dogs, Kaplan and Chaikoff (1935) found that the most notable increase in the livers, as compared

with normals, was in size, due largely to fat and water. Total weight of the liver was 5-7.5 per cent of body weight, whereas in normals it was about 5 per cent. Liver cholesterol in normal animals amounted to less than 1 gram, of which 17-20 per cent was esterified. In depancreatized animals, it amounted to 2-5 grams, of which 45-88 per cent was esterified. Phospholipid percentage, taking into account the extra fat and water, was practically unchanged. The accumulation consisted mainly of neutral fat and cholesterol esters. These authors (Kaplan and Chaikoff, 1937) found that both choline and raw pancreas would prevent fatty livers, but both took a long time to cure the fatty livers when once established. The accumulation of fat in the liver seemed to go along with a lowering of blood lipids (as though the "removal tendency" for blood fat was more pronounced than usual). Choline did not raise blood lipids over the normal level, while a heat-labile factor in pancreas did.

Kidney

Turner (1931) found a relatively high lipid content in cat's kidney as compared with kidneys of other animals, confirming the earlier work of Mottram (1916). The total fatty acid content varied from 2.1 to 6.9 per cent, averaging 4.9 per cent, with an iodine number of 63 to 113. Mottram's values were about 5 per cent total fatty acid, with an iodine number of 56. Beef kidney was found to average 2 per cent total fatty acids, with an iodine number of 134; human kidney, 1.9 per cent with an iodine number of 131. The phospholipid fatty acids of cat kidney had iodine numbers of 75 and 56 as compared with 95 for beef kidney. The acetone-soluble lipids yielded fatty acids with an iodine number of 57 to 64, while the same fraction from beef kidney gave an iodine number of 130. The fatty acids were found to consist of palmitic, stearic, oleic, linoleic, and at least two new liquid saturated acids.

Our own average values for beef kidney (Bloor, 1928) are given in Table 22 (p. 198). The values for total fatty acid percentage of moist tissue come within the range of those of Turner (1931) for beef kidney, but the iodine number is lower than Turner obtained. It is believed that too much emphasis should not be put on the iodine number, since it has been conclusively shown by Sinclair (1931) that the nature of the phospholipid fatty acids of tissue, including the degree of unsaturation, is directly affected by the fat of the diet, and that the kidney is one of the tissues which shows the effect quickly. Later, Haven (1935) showed the same to be true for tumor tissue.

Adrenal glands

Borberg (1915) found, in the adrenal cortex, cholesterol esters and

free fatty acids, mostly of the more highly unsaturated series, but no triglycerides. The fatty acids of the adrenals were investigated by Ault and Brown (1934), who found that the principal unsaturated acids were oleic and arachidonic, the tissue being very rich in the latter acid (22 per cent); the oleic acid was about 40 per cent. The saturated acids were palmitic, 23.8 per cent; stearic, 11.1; arachidic, 2.0; and myristic, 1.2 per cent.

Chauffard and his school (1920) believe that the suprarenals are connected with cholesterol metabolism (synthesis and esterification). Baumann and Holly (1923) question whether the adrenals are the source of cholesterol or even act as stores, since they found no significant change in cholesterol or lecithin in blood after successful partial or complete adrenalectomy up to the time that animals were moribund. These findings were confirmed for cholesterol by Randles and Knudson (1928). On the other hand, adrenalectomy was found by two groups of investigators (Joelson and Shorr, 1924; Yeakel and Blanchard, 1938) to bring about a hypocholesterolemia and a lowering of the other blood lipids, a finding which was disputed by Randles and Knudson (1928) for rats.

Bär and Jaffé (1925) reported that the suprarenal cortex in rabbits contained only phospholipids and cerebrosides. Cholesterol compounds were rarely present and then in minimal amounts. When rabbits were fed cholesterol, large amounts were found in the suprarenals and ovaries. Sorg and Jaffé (1924) found the suprarenal capsules free from cholesterol esters. Leulier and Dechanet (1926) found that while the cholesterol content of the spleen, liver, and lungs was constant from the first week of life, that of the suprarenals was high in the first week and increased thereafter. Parhon and Cahane (1931) reported that the quantities of cholesterol in the suprarenal cortex of different animals were markedly different. In the cow and sheep, they found 4.5 grams per 1000 grams; in the guinea pig, 35 to 67 grams per 1000 grams; in the cat, 109 grams per 1000 grams. In general, the cholesterol content was in inverse proportion to that of water. Brown and associates (1937) found the values for beef adrenals given in Table 31. No neutral fat was found.

Table 31. Lipid Analyses of Cortex and Medulla (Brown and associates, 1937).
(Per Cent of Moist Weight)

	Cortex	Medulla
Phospholipid	3.09	2.66
Total fatty acid	1.76	1.46
Free cholesterol	0.230	0.300
Total cholesterol	0.255	0.354
Ester cholesterol	0.025	0.054

Average values, in per cent of moist weight, obtained in this laboratory

for normal guinea pig adrenals by Whitehead, Oleson and Bloor (unpublished data) were as follows:

Phospholipid	Free Cholesterol	Cholesterol Ester	Neutral Fat	Total Lipid
4.03	0.38	2.87	8.56	15.84

The connection between the suprarenals and cholesterol metabolism is thus not clear. Regarding the general relations to the nervous system, Gibbs (1925) called attention to a possible connection between the suprarenals and the brain in fetal life in human beings. The suprarenals in the fetus are relatively very large, have a rich blood supply, and are glandular in nature. After birth they rapidly become smaller. When the cerebral hemispheres are absent, they are absent too. In anencephaly, the suprarenal is very small, probably indicating too rapid an evolution. The fetal suprarenal develops to a maximum relative weight at about the third month, and the lipid granules appear early. No great accumulation of lipids occurs, however, until about the sixth month, and this is coincident with the increase in lipid content of the brain at about this time.

The effect of anemia on the lipid content of the adrenals was studied by Marino (1933). Hemorrhage produced at first a diminution of free and combined cholesterol, with increase of phospholipids and fatty acids. During blood regeneration, there was an increase of all lipids. Hemolytic poisons produced at first an increase of free and combined cholesterol and fatty acids with no constant variation in phospholipid. During hemolysis, there was a diminution of the cholesterol and an increase of phospholipid and fatty acids, while during regeneration, the low free cholesterol level persisted, but all the other lipid fractions increased. Andersen and Sperry (1937) found, in the reproductive cycle in the rat, that the free cholesterol of the adrenals was stable and constant. The ester cholesterol was lower in spayed, pregnant, and parturient rats than during estrus, diestrus, and lactation. No relation was found between serum and adrenal cholesterol.

Spleen

The spleen of a yearling cow was found by Pfeiffer (1931a) to have a content of 94.6 mg. of total cholesterol per 100 grams of moist tissue, of which two-thirds was free. Fresh spleen pulp was found by Bouisset and Soula (1932) to contain 2.9 per cent of total lipids, of which 91 per cent was soluble in ether. Lignoceryl sphingosine was found by Tropp and Wiedersheim (1933) in spleen in amounts of 0.016 to 0.034 per cent of moist weight. No other ceramid was found and no cerebroside. The water content was 79 per cent. The spleen is a preferred place of deposit of lipids in those conditions of abnormal lipid metabolism known as

Gaucher's disease, Niemann-Pick and Schüller-Christian diseases (which see, p. 232).

Bone marrow

Glikin (1907) found that the lecithin content of bone marrow varied with the age of the animal, being very high in the young and decreasing or even disappearing with age, a finding confirmed by Bolle (1910). Cheng (1931) found that the gelatinous marrow contained 20 to 22 per cent more unsaturated fatty acid than the yellow marrow. The saturated acids were mainly palmitic and stearic; the unsaturated mainly oleic with some arachidonic. The phospholipid was higher in calf marrow than in that of adults. There was evidence, from the molecular weight, of the presence of fatty acids with a larger molecule than arachidonic acid (C_{20}). The following results obtained by Bolle (1910) will give an idea of the range of content of phospholipid in bone marrow: beef, 0.46 per cent; pig, 0.43 per cent; sheep, 0.40 per cent; dog, 0.83 per cent; and cat, 1.16 per cent.

Reproductive organs

The lipids and especially phospholipid apparently play an important part in the processes of reproduction. Sorg (1924), for example, found phospholipids and cerebrosides in both the seminal cells and the interstitial tissue; in the latter they are deposition products, but in the seminal cells they are part of the cells themselves. Sinclair (1940) recorded the curious fact that the fatty acids of the testicle phospholipid do not change in response to (labeled) fatty acids in the food, in that respect differing from all other tissues, even the brain.

The relation of lipid composition to physiological activity in the ovaries of pregnant rabbits was investigated by Boyd (1935). His conclusions were that up to the fourteenth to sixteenth day of normal pregnancy, phospholipid and free cholesterol increased, the maximum increase being 300 per cent, but that cholesterol ester and neutral fat changed little. At about the middle of pregnancy, phospholipid and free cholesterol began to decrease and cholesterol esters and fat to increase. Phospholipid and free cholesterol continued to fall until at term they were at the same level as at the beginning of gestation. The cholesterol ester value did not fall until the last day, and neutral fat remained high. This cycle of changes is very similar to that undergone by the corpus luteum (see p. 223). These results were interpreted to mean that the ovary reached the height of its activity about the middle of the pregnancy and then declined, presumably reaching the low point at term. In guinea pig ovaries, on the other hand, there was relatively little change (Boyd, 1936).

In the ovaries of *Rana pipiens*, Boyd (1938) found a two- to seven-fold increase in total lipids, neutral fat, total fatty acids, cholesterol both free and bound, and phospholipid during the production of ova.

Workers with the corpus luteum have found the usual fatty constituents characteristic of all organs: phospholipids, cholesterol (free and combined), and glycerides. Fenger (1916) found that it contained about fifteen times as much phospholipid as muscle and about the same percentage as the other endocrine organs except thyroid. Cartland and Hart (1925) found in the acetone-soluble fatty portion about 52 per cent of the fatty acids present as glycerides, the rest as fatty acids or soaps. Of the total fatty acids, about 35 per cent were saturated and 65 per cent unsaturated. The fatty acid mixture consisted of: palmitic, 25 per cent; stearic, 11 per cent; oleic, 33 per cent; linoleic, 17 per cent; and arachidonic, 8 per cent; the volatile acids were represented by caproic and caprylic. Cholesterol, free and combined (palmitate), was present in a large amount. Hart and Heyl (1926) found in the lecithin from corpus luteum, palmitic, arachidonic, and oleic acids, and a three-double-bond acid with twenty carbon atoms. Linoleic and linolenic acids were lacking, and the presence of stearic acid was doubtful. Some protagon was present. They found that the cephalin fraction was similar to that from the liver, heart, and brain, and consisted mainly of cephalin, some lecithin and some split products of the two. Levene and Komatsu (1919) had also found that the cephalin fraction of tissues ordinarily consisted of a mixture of cephalin and its decomposition products.

Chauffard, Laroche and Grigaut (1912) found that cholesterol increased continuously during the metabolic cycle of the organ and suggested that the corpus luteum was one of the places where cholesterol was formed, or at least stored. A study of the lipid content of the ovary from 120 human cases by von Mikulicz-Radecki (1923) showed that in the development of the corpus luteum, phosphatides and cerebrosides appear first, then cholesterol and cholesterol ester mixtures. Hermstein (1925) made a study of the lipids of the human corpus luteum and found the highest content of phospholipid to be at the time of full activity of the gland. Kaufmann and Raeth (1927) made an analytical examination of twenty-four human corpora lutea at various stages and found high lecithin at the functioning stage, the values falling markedly at the onset of menstruation. In pregnancy, the lecithin content increased to the end, but the cholesterol content did not change greatly. Boyd and Elden (1935) found that the estrin content of the corpus luteum was proportional to the free cholesterol. The other lipid fractions were found not to be related to the hormone concentrations.

The lipid changes in the corpus luteum of the sow throughout its cycle

has been followed by Bloor, Okey and Corner (1930), who found that the content of the different lipids varied markedly with the activity of the gland, the phospholipid content being two to three times as high during the period of activity (previous to estrus and during pregnancy) as at the time of growth or retrogression. Free cholesterol increased up to and during the period of active functioning, but to a much less extent. Cholesterol ester content, on the other hand, varied inversely with the activity of the gland, a high content being characteristic of the degenerated gland. These results agree with those reported by Kaufmann (1927).

The placenta was analyzed for lipids during various periods of pregnancy by Watanabe (1923), who found that the total lipid was higher in early pregnancy than later. Phospholipid constituted about 30 per cent of the total lipid and most of it was lecithin. Both free and bound cholesterol were present. Neutral fat constituted about 27 per cent of the total lipid.

The passage of lipids through the placenta has been shown to take place fairly readily: linseed oil fatty acids (Bickenbach and Rupp, 1931; Miura, 1937), cod liver oil fatty acids (Sinclair, 1933), elaidic acid (Chaikoff and Robinson, 1933; McConnell and Sinclair, 1937a,b). Miura (1937) reported that linseed oil raised the iodine number of the fatty acids of both mother and fetus, but that coconut oil lowered it, thus showing the passage of fat through the placenta. Linseed oil and elaidin affect the phospholipids of both mother and fetus; coconut oil affects them less. The passage of lipids from the mother to the fetus through the placenta was followed by McConnell and Sinclair (1937a,b) with a labeled fatty acid (elaidic). It passed readily into the fetus through the placenta in rats to the extent of 11 per cent of the total fatty acids of the entire body of the newborn young. It also passed into the rat milk, so that after 10 days of suckling by mothers on a diet rich in elaidic acid, the elaidic acid made up 61 per cent of the total fatty acids of the animal.

From their study of the lipid composition of blood from the umbilical artery and vein in the human fetus, Boyd and Wilson (1935) conclude that phospholipid and cholesterol are regularly absorbed by the human fetus from the umbilical blood at birth, also ester cholesterol if there is sufficient, and that neutral fat may be either absorbed or given up. The cholesterol so absorbed is retained as such; the phospholipid is hydrolyzed and the phosphorus retained by the embryo, and the fatty acids may be returned to the umbilical blood as fat. It was estimated that over 40 grams of lipid, of which 75 per cent was phospholipid, was absorbed by an average human fetus at birth. The fetus appears to absorb more of the saturated phospholipid and has a special avidity for cephalin. The selec-

tion of the saturated phospholipids fits in with the laying down of a relatively hard depot fat, as noted by Cattaneo (1932).

Cattaneo (1932) examined the ether-extracted fat of the human fetus and found that it had a solidifying point of 22-25°C. The iodine number of the fat was 45.9-51.5 and its average molecular weight was 241. It was a definitely lower-melting fat than the fat of the pregnant woman. Its properties are probably due to the fact that it was laid down in a region of the mother's body in which the temperature was relatively high, and, therefore, according to the Henriques-Hansen rule, it would have a relatively high melting point and a high content of saturated acids.

Hentschel (1932) found that the total sterol content of the fetus increased throughout the period of intrauterine development up to the time of birth, while the relative content became stable at the margin of viability. In the brain, development and sterol content ran parallel. The skin showed its highest relative total sterol content in the earliest developmental stages.

Skin and appendages

Skin. Analyses of the exfoliated scales of skin were made by Eckstein and Wile (1926). They found cholesterol from 0.58 to 1.5 per cent of the skin, with 90 per cent of it free. It constituted 13 to 24 per cent of the total lipid. Phospholipid was 2.5 to 3.15 per cent of the total lipid. Roffo (1928) examined the cholesterol content of the skin in various parts of the body and found much more in the exposed parts (face) than in the unexposed parts (abdomen). Exposure to sunlight increased the cholesterol content. He found a relationship between cholesterol content and frequency of skin tumors.

Koppenhoefer (1936) divided the skin (steer hide) into six layers (hair, epidermal horn, epidermal base, transition, corium, and corium base) and determined their lipids separately. The values obtained were as follows (Table 32):

Table 32. Lipid Distribution in Fresh Steer Skin (Koppenhoefer, 1936). (Per Cent of Dry Weight)

	Hair	Epidermal Horn	Epidermal Base	Transition	Corium Major	Corium Base
Cholesterol	0.67	1.01	0.97	0.22	0.06	0.08
Phospholipid	0.0	0.52	1.97	0.17	0.08	0.05
Wax	0 97	2.21	2 55			
Free fatty acids	1.24	2.09	0.49	0.21	0.06	0.15
Acetyl value of total fatty acids	40.7	47.0	56.8	37.8	2 4	1.4

Wax, phospholipid, and cholesterol were high in the epidermal region. The basal epidermal layer was most active metabolically. The sebum, formed from the degenerating nucleated cells of the glands, contained

cholesterol esters, and waxes of hydroxy acids with aliphatic alcohols. Degenerated skin lipids yielded oxidized cholesterol and free fatty acids which became saturated and hydroxylated. In the corium, there was mostly fat, containing 65 per cent liquid and 35 per cent solid fatty acid. The liquid acid was oleic, the solid palmitic and stearic. The cephalin had fatty acids of greater unsaturation and higher melting point than the lecithin, which contained entirely liquid acids.

Engman and Kooyman (1934) found that the fatty substances of the skin surface, arising from the sebaceous glands, sweat glands and epidermal cells, contained a large amount of unsaponifiable matter—27-36 per cent of the total lipids. Sterols constituted two-thirds of this unsaponifiable of the stratum corneum and less than half of the superficial lipids of the skin. There was a high content of free fatty acids.

Irradiation of the skin with sunlight was found to increase its cholesterol content and keeping animals in the dark was found to decrease it (Roffo, 1931). In the adult (Roffo, 1932), there was much more cholesterol in the skin of the face than in that of the back. In the infant or fetus, this was not true, but in the infant the cholesterol of the facial skin increased with age, because of the effect of light.

Hair, feathers, wool. Eckstein (1926) found in the hair of young adult albino rats 4.5 per cent total lipid, of which 12 per cent was cholesterol made up of 80 per cent free and 20 per cent ester. Phospholipid constituted 0.8 per cent of the total lipid. In Table 33 Eckstein (1927) gives analyses of hair, wool, etc.:

Table 33. Percentage of Lipids and Cholesterol in Hair, Wool and Feathers (Eckstein, 1927).

	Total Lipid	Free Cholesterol	Total Cholesterol
Hair			
Human adult*	6.1	0.08	0.15
Human child*	3.6	0.23†	0.36
Rabbit*	1.5	0.53	0.57
Rat	4.3	0.41	0.57
Cat	5.3	0.46	0.55
Dog	1.8		0.56
Wool*	10.8	0.47	0.82
Feathers*	2.0	0.18 (duck)	0.28
Quills*	3.5		0.35

* Average value.
† Value for 10-13 year old children omitted in getting this average.

The following values for rabbit hair are recorded by Kawaguchi (1937): Cholesterol content averaged 448 ± 273 mg. per cent, lower in spring and summer, higher in autumn and winter. The younger the rabbit, the higher the cholesterol content. Color of hair made no difference. Curly hair had a lower content than straight hair.

Salgues (1937) analyzed the feathers of twelve species of wild birds. Those from carnivorous birds contained more fat and protein and less ash than those of graminivorous birds. Cholesterol ranged from 0.06 to 0.54 per cent, being higher in young birds.

Drummond and Baker (1929) examined the lipids extracted from merino wool with petroleum ether. There were small amounts of free fatty acids but no glycerol, and the extract consisted mainly of fatty acid esters of the higher aliphatic alcohols, cetyl and ceryl, and of cholesterol and iso-cholesterol. The fatty acids found were cerotic, stearic, and palmitic.

Gums. The total lipid and phospholipid content of the gums lies between the values for skin and intestinal mucosa (Hodge, 1933), averaging for cholesterol 0.20 ± 0.08 per cent of moist weight, phospholipid 0.95 ± 0.74 per cent, total lipid 2.15 ± 1.65 per cent, with somewhat lower values for infants than for adults. There was no correlation between lipid content and the health of the mouth.

Gastric mucosa. Uhnoo (1938) found lecithin, cephalin, and small amounts of cerebrosides in the gastric mucosa.

Intestine. Bürger and Oeter (1929a) reported for the upper intestine in humans a content of about 0.37 per cent of cholesterol; for the sigmoid 0.65 per cent. For the large intestine (Bürger and Oeter, 1929b), 100 grams dry weight of whole large intestine contained 0.65 gram of cholesterol while the same amount of mucous membrane contained 0.82 gram. In terms of the mucosa of the whole large intestine, this would amount to a total of only about 78 mg., and indicates that the mucosa can give off daily in the form of excretions more cholesterol than it contains. No cholesterol ester was found in any part of the large intestine.

The content of phospholipid in the intestinal mucosa has attracted a good deal of attention because of its connection with fat absorption. Sinclair (1929) presented evidence to show that the fatty acids of the food fat are transformed into phospholipid in the intestinal mucosa and liver within a few hours after ingestion. Verzár and Laszt (1934) confirmed the participation of phospholipid in fat absorption and reformation of fat in the mucosa.

Eyes: lenses. Salit (1931) gives the following analyses of the lipids of lenses of animals and of human eyes with cataracts in milligrams per 100 grams:

	Cholesterol	Phospholipid	Total Fatty Acids
Cattle (1½ to 2 years old)	43	109	
Rabbit	51	131	
Human with cataract (av.)	610	990	2800

Notable is the very high cholesterol in the cataractous eye, which agrees with the findings of Updegraff (1932).

Analyses of various tissues of the eye have been made by Krause (1934). These tissues (sclera, cornea, choroid, and iris) show a moderate content of lipids, only the cornea reaching 2 per cent. Of the phospholipids, lecithin and cephalin are present in largest amount, and sphingomyelin in small amounts except in the cornea, where it is notably high. Cerebrosides are present in small amounts and cholesterol in larger amounts, especially in the epithelial portion of the cornea, in the choroid, and in the iris. In the retina, the total lipid reaches 2.24 per cent of the moist weight and consists of about 70 per cent phospholipids; cholesterol is about 0.23 per cent. The vitreous humor (Krause, 1935) contains very little lipid, less than 0.014 mg. per cent, with cholesterol less than 0.012 mg. per cent.

In 44 human lenses, Bunge (1938) found that total cholesterol increased with age, amounting in the aged to five times that in infants. About one-sixth of the total cholesterol was in the ester form. Incipient and intumescent cataract did not mean higher cholesterol.

Gall bladder

The walls of the gall bladder were found by Mentzer (1925) to have the power to absorb lipids, probably by direct passage through the epithelial cells of the mucosa; 46 per cent of persons over 20 years had gross evidence of accumulated lipid in the gall bladder walls.

Human fat

Cathcart and Cuthbertson (1931) took samples from fourteen subjects ranging from 18 to 57 years of age. Cholesterol and lecithin were removed before analysis and corrections made for the presence of cholesterol esters and for nitrogen and phosphorus compounds that were not removed. Fat from the panniculus adiposus abdominalis contained 76.16 per cent carbon, 11.82 per cent hydrogen, and 11.98 per cent oxygen. Its respiratory quotient was 0.711, calorie value 9.506 per gram, and iodine number 68.4. Fat from the liver, practically free of other lipids, contained 73.79 per cent carbon, 11.42 per cent hydrogen, and 14.36 per cent oxygen. Its respiratory quotient was 0.717, calorie value 9.23 per gram, and iodine number 74.

Cold-blooded animals

Terroine and co-workers (1930) made a study of the degree of unsaturation of the fatty acids in the tissues of cold-blooded animals. They found that the fatty acid content of the phospholipids of the tissues

of these animals is always low, averaging about 60 per cent; that the iodine number of the phospholipid fatty acids is not as constant as in the warm-blooded animal; and that the phospholipid fatty acids have an iodine number generally below that of the stored fat (in warm-blooded animals the reverse is the case), but higher than in the corresponding tissue of the warm-blooded animals. An analysis of the fat of the boa gave the following (Kerr, 1927): melting point 28.5°C., saponification number 197, free fatty acids 0.17 per cent, and iodine number of liquid fatty acids 113. The saturated acids consisted of palmitic and stearic; the unsaturated of oleic, linoleic, and a four-double-bond acid.

The fat of grasshopper eggs was examined by Slifer (1932), who found that the iodine number of the fatty acids from the eggs of seven species of grasshopper varies from 128 to 167. The temperature of complete fusion of the fatty acids depends upon the breeding habits. Eight species which lay eggs in the fall to hatch the following spring gave fatty acids melting in the range 25.5 to 30.5°C. In the three species which lay eggs in the spring to hatch during the summer the fatty acids melted in the range 37.0 to 39.5°C. Factors other than environment play a leading role in determining the type of fat in the eggs of *Acrididae*. Timon-David (1930) found that the larvae of 24 insects contained 0.94 to 28.0 per cent fat. The iodine numbers vary from 1.2 in aphidians to 164 for *Saturnia pyri*. Linolenic acid was found (determined as hexabromstearic) and oleic acid was widely distributed. Unsaponifiable varied from 0.75 per cent to 12.5 per cent. Oils of tropical insects had a lower iodine number than those in cold countries. Females were richer in fat than males.

In *Cysticercus fasciolaris*, Salisbury and Anderson (1939) found lipids consisting of phospholipids (lecithin and cephalin), cholesterol, cerebrosides, and a small amount of neutral fat. The phospholipid comprised about 30 per cent of the lipids and its fatty acids contained equal parts of saturated (palmitic, stearic, and arachidic) and unsaturated acids (oleic and palmitoleic).

The lipids of grasshopper muscle (*Brachystola magna*) were examined by Stoneburg (unpublished data) in this laboratory. Phospholipid in thoracic muscle was 1.025 per cent, in the thigh muscle 1.24 per cent, cholesterol 0.045 per cent of the moist weight.

Tissue Lipids in Abnormal Conditions

A large proportion of the investigations on the tissue lipids in abnormal conditions has been concerned with those conditions which involve obvious deposits or abnormal growths in chronic conditions. Very little work has been done on the tissues of acutely ill animals, although

Table 34. Lipid Content of Human Tissues as Reported in the Literature (Cowie and Magee, 1934).

(Grams per 100 Grams of Fresh Tissue)

Tissue	Age	Reporter	Pathologic Diagnosis	Total Lipid	Phospholipid	Cholesterol
Liver	8 mos. fetus	1	....	3 34	..	0.285
	5½ yrs.	1	Pulmonary and meningeal tuberculosis	4 44	....	0.341
	11 mos.	1	von Jaksch's anemia	2 85	....	0.180
	22 yrs.	8	Normal	3 04	1.79	.....
	35 yrs.	8	Normal	3 45	1.71	.....
	Died at birth	8	....	5 16	1.71	.....
	20 yrs.	4	Normal	....	....	0.324
	29 yrs.	4	Normal	....	....	0 377
	30 yrs.	4	Normal	....	....	0.254
	10 mos.	2	Secondary anemia (fatty degeneration of the liver)	8 09	1 79	0.280
	2 yrs.	2	Schuller-Christian's disease	3.17	2 24	0.398
	3 yrs.	7	Schüller-Christian's disease	4.46	0.291	1.060
Spleen	3 mos. fetus	1	....	1.95	....	0 368
	8 mos. fetus	1	....	1 83	....	0 306
	6 yrs.	1	Polyarthritis; secondary anemia	3 284	....	0 235
	Infant	7	Normal	1 108	0 297	0 162
	10 mos.	2	Secondary anemia	3 64	1 43	0 337
	3 yrs.	7	Schüller-Christian's disease	1 07	0 254	0 794
	2 yrs.	2	Schüller-Christian's disease	2 36	1.53	0 286
Lungs	3 mos. fetus	1	....	1 47	....	0 188
	8 mos. fetus	1	....	1 30	....	0 166
	10 mos.	2	Anemia; terminal bronchopneumonia	4 62	1 33	0 335
	2 yrs.	2	Schüller-Christian's disease	3 01	1 69	0.432
Kidneys	....	9	Normal	2.06	....	0 272
	....	9	Normal	1 87	...	0 250
	8 mos. fetus	1	....	2.87	....	0 255
	6 yrs.	1	Polyarthritis; secondary anemia	4 25	....	0 134
	11 mos.	1	von Jaksch's anemia	3 17	....	0.199
	20 yrs.	4	Normal	....	....	0.310
	29 yrs.	4	Normal	....	....	0 340
	30 yrs.	4	Normal	....	....	0 293
	10 mos.	2	Secondary anemia (fatty degeneration of the liver)	4 02	1 14	0 362
	2 yrs.	2	Schüller-Christian's disease	2 40	1 33	0.250
Suprarenals	8 mos. fetus	1	....	2 90	....	0 358
	6 yrs.	1	Polyarthritis; secondary anemia	14 626	....	1 099
	20 yrs.	4	Normal	....	....	6 664
	29 yrs.	4	Normal	....	....	2 595
	30 yrs.	4	Normal	....	....	5 003
	2 yrs.	2	Schüller-Christian's disease	7 07	1 25	2 08
Pectoral muscle	....	6	Normal	8 74	0 667	0.351
		6	Normal	7 28	0 53	0 377
Rectus abdominis muscle	10 mos.	2	Secondary anemia (fatty degeneration of the liver)	5 90	0 49	0.121
	2 yrs.	2	Schüller-Christian's disease	0 90	0 47	0 128
Psoas muscle	2 yrs.	2	Schüller-Christian's disease	0.84	0.47	0 110
Heart muscle	3 mos. fetus	1	....	3 91	....	0.193
	8 mos. fetus	1	....	3 10	....	0 173
	10 mos.	2	Secondary anemia (fatty degeneration of the liver)	4.19	1.43	0 118
	2 yrs.	2	Schüller-Christian's disease	2.66	1.82	0.174
Bone marrow	16 mos.	5	Bronchopneumonia	8 00	1.99	.....
	7 mos.	5	Pneumonia	2.48	1.52	.....
	61 yrs.	5	Influenza	68 4	1.38	.....
	8 mos. fetus	1	....	12.264	....	0 056
	2 yrs.	2	Schüller-Christian's disease	3.013	1 04	0 664
Xanthomas	....	3	From dura mater	9.554	0.45	5 119
	3 yrs.	7	From dura mater	10 39	0.72	4.23
	2 yrs.	2	From dura mater	6.96	0.83	3 462
	2 yrs.	2	Of bone marrow	7.386	1.59	4.023

Reporter

1. Beumer, H., (1921).
2. Cowie, D. M. and Magee, M. C., (1934).
3. Epstein, E. and Lorenz, K., (1930).
4. Fex, J., (1920).
5. Glikin, W., (1907).
6. Jowett, M., (1931).
7. Kleinmann, H., (1931).
8. Theis, E. R., (1929).
9. Windaus, A., (1910).

this type of investigation often produces tangible results. For example, Gérard, Moissonier and Welti (1932) give values on the lipid change in the tissues of monkeys with yellow fever. The most constant finding was a lowered phospholipid content of all tissues. Unsaponifiable matter was lowered in all tissues but the spleen, and cholesterol in all tissues but the spleen and liver. The effect of continuous feeding of alcohol (0.9-2.5 grams per kilo per day) for several months with occasional rest periods was investigated (Sieber, 1910), with the finding that all organs except the kidney showed much lowered phospholipid. In general, the lowering was about half of the normal value. A review of the literature on the lipid content of human tissues is given in Table 34 from Cowie and Magee (1934), who collected the data in connection with their work on lipid diseases. Presumably they represent all or most of the tissue analyses available before 1934.

Diseases involving lipid deposits

A series of abnormal conditions or diseases in which the lipids are involved has attracted a good deal of attention. The cause of the diseases seems to be a failure in the utilization of the deposited substances, which are then collected by the scavenger systems of the body and stored. The liver, spleen and the reticulo-endothelial system in general get most of the material, but any tissue which is below par in a metabolic sense, for example the arterial walls, may become the depository. The diseases referred to are: Gaucher's disease, characterized by enlargement of the liver and spleen which contain deposits of cerebrosides, mainly cerasin; the Niemann-Pick disease, characterized by accumulations in the liver and spleen of phospholipid, mainly lecithin and sphingomyelin; and the Schüller-Christian disease, characterized by deposits of cholesterol and its esters in the skin, mucous membrane, and inner organs. Since most of the information concerning them is in pathological rather than in chemical terms, those interested are referred to a review of these conditions by Rowland (1927-36); only brief references will be given below. In addition, there are other conditions which have been known for a longer time: amaurotic idiocy is characterized by lipid deposits in the brain, spleen, liver and kidneys. Lipid deposits have been found in cysts, and deposits of cholesterol and cholesterol esters are prominent in arteriosclerosis and in a condition sometimes known as cholesterol gout.

An instance of familial lipemia including enlarged and fatty liver and spleen was reported by Holt, Aylward and Timbus (1937). Blood fat up to 7 per cent was found, and there was a tendency to hypertrophy of the liver and spleen with accumulation of fat.

The skin nodules in leprosy were analyzed by Paras (1938), who

found in per cent of dry weight: phospholipid 3.2, acetone-soluble 6.4, and wax 1.5.

The blood in thirty kinds of skin disease was examined by Rosen and Krasnow (1932) who found that the cholesterol ranged from a low figure to one that was high and variable in xanthoma (232 ± 153 mg. per cent) and in acne vulgaris (158 ±14). Phospholipid extended from 244 ± 13 for the acne group to 286 ± 60 for the xanthomas. Chanutin and Ludewig (1937) found increased free cholesterol, phospholipids, and total lipids with reduction in cholesterol esters and fat in blood plasma in xanthomatosis associated with hepatic damage.

Schüller-Christian disease. Kleinmann (1931) made analyses of the organs in a case of Schüller-Christian disease. In the spleen, the cholesterol-to-lecithin ratio was 2.7 to 1, in marked contrast to that recorded in Niemann-Pick disease, 1 to 9.3, as well as to that observed in normal spleens. Cholesterol as esters greatly surpassed free cholesterol. Similar relations to those noted for the spleen were found in the dura, liver, and subcutaneous fatty tissue (see also Cowie and Magee, 1934).

Gaucher's and Niemann-Pick diseases. Teunissen (1937) found, in the livers and spleens of Niemann-Pick disease, the lecithin and sphingomyelin greatly increased (5-10 times normal), but in Gaucher's disease the increased lipid of the spleen was nearly all cerasin. Cholesterol was greatly increased in the liver of both, but not in the spleen. Sobotka and associates (1933), in Gaucher's and Niemann-Pick diseases, found lipids in the spleen up to four times normal values, with cholesterol esters much increased, phospholipid not greatly changed, fat increased somewhat, and cerebrosides greatly increased. The livers had a higher than normal lipid content with increased cholesterol, but other lipids were not greatly different. Halliday and associates (1940) found that the cerebroside (cerasin) from the spleen of a case of Gaucher's disease contained dextrose instead of galactose, which is the usual carbohydrate constituent. Klenk (1935) and Tropp and Eckardt (1936b) found that most of the phospholipid in the spleen and liver in Niemann-Pick disease was sphingomyelin. In the brain also (Klenk), the sphingomyelin exceeded the glycerophospholipids, and the high sphingomyelin was matched by an almost complete absence of cerebrosides. The liver sphingomyelin contained lignoceric, nervonic, stearic, and palmitic acids, and that from the brain contained almost pure stearic. There was therefore a lack of the normal C_{24} acids in the brain. The liver glycerophospholipids had their usual complement of C_{20} and C_{22} acids, but those of the brain did not.

Cholesterol gout. In addition to these are less well-defined types in which the principal symptom is the deposition of cholesterol and cholesterol esters in nodules in the skin and in various other places such

as the joints. These deposits are similar to those of uric acid in gout, and the condition has been called cholesterol gout. In one case it was treated with atophan (Gaál, 1930). The condition appears to be due, at least partly, to an inability, often congenital, to excrete cholesterol in normal fashion. Arning and Lippmann (1920) demonstrated a high blood cholesterol which was increased by feeding cholesterol, the effect of which persisted for a long time after the feeding. The excess cholesterol was deposited in various tissues, sometimes in the valves of the heart. Schoenheimer (1933) made a study of a typical case of this condition with deposits of cholesterol in the joints and tendons and as nodules in the skin. The serum cholesterol was 852 mg. per cent and the total lipid 2.1 per cent; at the same time 93 per cent of the cholesterol was present as ester. The content of the corpuscles was normal. Dihydrocholesterol was present in the plasma to the extent of 9.8 per cent of the sterol (normal 2-3 per cent of the sterol). This patient excreted only about 113 mg. cholesterol daily as compared with 800 mg. in a normal person. On a cholesterol-free (vegetable) diet, the blood cholesterol fell to 350 mg. per cent in 50 days. The accumulation of cholesterol in the blood resulted in the deposition in the tissues. The behavior of these individuals is similar to that of herbivorous animals, such as the rabbit, which are unable to excrete cholesterol sufficiently fast when it is fed in excess. In this connection it is interesting to note that alcohol fed with cholesterol to rabbits has been reported by Eberhard (1936) to reduce somewhat the tendency of blood cholesterol to deposit in the tissues, probably by slightly increasing its solubility in the blood.

Arteriosclerosis and atheromatosis. Small (1916), in a review of the role of cholesterol in atheromatosis, reported that cholesterol ester deposits occur at sites of low-grade chronic inflammation, and cholesterol crystallizes out where there is slow cell destruction and absorption is poor. Feeding of cholesterol to rabbits (and presumably to other herbivorous animals in which a mechanism for disposal of extra cholesterol has not been well developed) results in a deposition of cholesterol and cholesterol esters in the arterial walls closely simulating atheromatosis in man. This early work has been confirmed by Duff (1936), who, after feeding cholesterol for 8-119 days, found a deposition of cholesterol esters in both the intima and media of the aorta. These depositions took place most readily at injured areas. While it is probable that results obtained with rabbits have little or no bearing on what happens in an omnivorous animal with a well developed mechanism for disposing of extra cholesterol, the possibility still exists that a continued large ingestion of cholesterol by human beings when there is already a high level of blood cholesterol may be a factor in the production of arteriosclerosis.

especially in the presence of tissue injury or weakness which would increase the receptivity of the tissues. However, Landé and Sperry (1936) could find no correlation between the level of blood cholesterol and the high lipid content of the aorta and therefore of the degree of arteriosclerosis.

The relation between arteriosclerosis in man and the presence of lipids in the vessel wall has been investigated by Schoenheimer (1926, 1928). In sclerotic arteries he found a large accumulation of cholesterol esters. Whereas in the normal aorta the cholesterol and cholesterol esters amount to 20 to 60 mg. per cent, in arteriosclerosis the values may reach 100 to 113 mg. per cent. There was no relation between the extent of calcification and amount of cholesterol esters. The amount of phospholipid in the artery wall was quite small (maximum 5.7 per cent of the total lipid) but greater with increased lipid, and neutral fat was absent. The fatty acids in combination with cholesterol were stearic, palmitic, and oleic with recognizable amounts of more unsaturated acids. Galactosides were present.

Labbe, Nepveux and Heitz (1923) found, in patients with the circulation intact, that the arterial wall contained 1/3 mg. of cholesterol per gram of wall substance; but in a series of cases with a more or less extensive development of arteritis obliterans the quantity rose as high as 22.7 mg. Hypercholesterolemia was considered of importance diagnostically as signifying the onset of a general arteritis obliterans.

Cysts. In a dermoid cyst, Dimter (1932) found as follows: The ether extract contained about 60 per cent of unsaponifiable substance, including 6.4 per cent free and 20.3 per cent ester cholesterol. The lecithin content (lipid phosphorus) was 0.56 per cent. Cerebrosides constituted 7.5 per cent. There was about 20 per cent of a hydrocarbon which was either squalene ($C_{30}H_{50}$) or some closely related substance. Behmel (1932) found dihydrocholesterol and no highly unsaturated sterols.

In a frontal cyst of about 300 cc. volume, Tropp and Eckardt (1936a) found no trace of blood pigment and no cholesterol, but a high sugar value after hydrolysis. The main constituent was a mixture of cerebron and cerasin in about equal amounts.

Tumors. The relation of the lipids to tumor formation and growth has engaged the attention of many workers. Bullock and Cramer (1914) compared slowly growing and rapidly growing tumors in mice. They found total lipid to the extent of 13.1 per cent of the dry substance in both; cholesterol was 1 per cent of the total solids in slowly growing but present only in traces in rapidly growing tumors. Phospholipid was small in amount in both, but greater in the rapidly growing tumors.

Neither contained cholesterol esters or cerebrosides and most of the lipid was fat. Comparing sarcoma and carcinoma, they found phospholipid to be 40 per cent of the total lipid in sarcoma as compared with 7 to 10 per cent in the carcinoma. Cerebrosides were present in the sarcoma (2 per cent of dry substance) but there were none in the carcinoma. It has been found by Bierich, Detzel and Lang (1931) that malignant tumors have a higher content of phospholipid and cholesterol than benign tumors, or than that of the tissue surrounding the tumor. The same has been found in this laboratory by Yasuda and Bloor (1932). In the tumors examined by them, which included those of humans and rats and mice, the phospholipid and cholesterol content averaged about twice as great in the malignant tumors as in the benign (Table 35). Jowett (1931) found that pure malignant tumors were higher in phospholipid and cholesterol than tumors mixed with normal tissue.

Table 35. Lipid Content of Tumors* (Yasuda and Bloor, 1932). (Grams per 100 Grams of Dry Tissue)

Tumors	Dry Substance (%)	Phospholipid	Cholesterol† Total	Cholesterol† Free	Fat	Residual Unsaponifiable Content	Residual Unsaponifiable % of Total Unsaponifiable
Tumors other than cancer	19.57	3.15	1.05	0.93	1.42	0.22	25.8
Human cancers	20.09	5 27	1.61	1.08	8 33	1 38	38.7
Mouse carcinoma	18.82	7.12	2.37	1.71	4.09	0.94	28 3

* Average values.
† Cholesterol was determined by the digitonin oxidative method.

Takizawa (1937) estimated the cholesterol content of the organs of chickens bearing transplanted chicken sarcoma and found high values in lungs, liver, spleen, pancreas, and muscle; low values in suprarenal and bone marrow; normal values in brain, heart, gastric gland, stomach, kidney, and testis.

Haven (1937a) fed a "labeled" fat (elaidin) to rats and studied its rate of incorporation into their tumors (carcinosarcoma 256). She found that elaidic acid entered the tumor to the extent of about one-fifth of the phospholipid fatty acids and that the rate of entrance was slow compared with its rate of entry into the liver phospholipid, leading to the conclusion that the phospholipids of tumors like those of muscle are mainly of the non-metabolic or structural type, rather than of the metabolic type on the way to combustion. The ratio of solid (saturated) to liquid (unsaturated) fatty acids of rat tumor is the same as that of rat muscle phospholipid, namely 30:70. Comparing the lipid content of the center and periphery in these tumors, Haven (1937b) found that the periphery (non-necrotic, growing tissue) had about twice the phospholipid content and much less cholesterol ester, free cholesterol, fat, and

water than the center (necrotic, non-growing tissue). The phospholipid of the actively growing tissue is apparently transformed into cholesterol esters and fat when the tissue ceases to grow, the fatty acids from two molecules of phospholipid yielding one molecule of fat and one molecule of cholesterol ester.* The rate of growth of these tumors is notably less when cod liver oil constitutes the fat of the diet than when it is coconut oil. The growth of the whole animal is the same with both fats (Haven, 1936).

Jones, Chaikoff and Lawrence (1939), using radioactive phosphorus, have found that the phospholipid metabolism of mammary carcinoma, lymphoma, lymphosarcoma and sarcoma 180 resembles in its rate of turnover that of the liver, kidney, and intestine rather than that of brain or muscle. This work has since been confirmed by Haven (1940) for Carcinosarcoma 256, the tumor used in previous work with elaidic acid. The difference in results obtained with the two indicators (elaidin and P^{32}) suggests that here is an example of replacement of parts rather than a synthesis of the whole molecule, and that the phosphoric acid component is more rapidly replaced than the fatty acids. Jones, Chaikoff and Lawrence (1940) found that when two or three different tumors were grown on the same animal each tumor maintained its characteristic phospholipid phosphorus turnover.

Nature and Function of the Tissue Lipids

Fat

Nature of stored fat

Sources. The characteristic functional lipids of organs and tissues of animals consist, as noted above, of phospholipids and cholesterol. The stored lipid is characteristically neutral fat with very little else; it represents, more or less directly, excess food, largely carbohydrate and fat and possibly some protein.

Protein. Formation of fat from protein is theoretically possible, since most of the protein molecule has been shown to yield dextrose in the diabetic states. It is probable, however, that very little fat is normally formed from protein, as very little, if any, of it normally escapes combustion because of its great stimulating effect on metabolism (specific dynamic action). For example, Atkinson and Lusk (1919) found that it was very difficult to get a dog to eat enough protein to show any considerable formation of fat. Whatever fat is formed from protein has, as Anderson and Mendel (1928) found, the same properties (iodine ab-

* The same process appears to take place in the incubating hen's egg at about the fourteenth day and the process in reverse seems to be characteristic of the removal of fat and cholesterol esters from fatty livers on feeding choline.

sorption values and refractive index) as that formed from carbohydrate, thus supporting the view that the formation of fat from protein takes place by way of a carbohydrate stage. The same result was shown later by Eckstein (1929) with purely synthetic diets.

Carbohydrate. The formation of fat from carbohydrate, first suggested by Liebig, is now universally admitted, both on experimental grounds and as the result of empirical practice in feeding animals. The proofs need not be detailed here, but some of the factors in the process are of interest. The respiratory quotient (R.Q. = vol. CO_2 output in the lungs/vol. O_2 intake) in animals fattening on carbohydrates is often above 1.0. Since the highest respiratory quotient found from the combustion of any foodstuff is 1.0 (carbohydrate), these high quotients mean that oxygen is being supplied from some other source than the respired air; and since the transformation of carbohydrate to fatty acid would liberate oxygen ($3C_6H_{12}O_6 = C_{18}H_{36}O_2 + 8O_2$), the assumption is that the extra oxygen comes from this source. The quotients found in rapid fattening are often quite high, as for example the value 1.58 reported by Wierzuchowski and Ling (1925) for a young hog. Bleibtreu (1901), in his study of the fattening of geese by forced feeding of rye, found quotients up to 1.38 and stated that the respiratory quotient could be kept continuously over 1.0. He found in these animals that the high values were mainly the result of increased carbon dioxide production (as the result of the specific dynamic action of the food), the oxygen intake being not much above what it was in the animals in the normal state. Milky serum was observed in many animals and this, since it disappeared on fasting or on a fat-free diet, he attributed to the oil of the rye, which could not easily be stored in the excessively fat tissues.

Wierzuchowski and Ling (1925), in their study of fat production from carbohydrate in young pigs, found that the specific dynamic action of carbohydrate doubled the heat production in these animals. The pig transformed carbohydrate to fat at a rate two and one-half times its basal caloric requirement, resulting in the formation of nearly 1 per cent of its body weight of fat per day. Respiratory quotients of 1.4 were common, and, as mentioned before, one quotient of 1.58 was found. In one experiment 20 hours after starch ingestion, the respiratory quotient was 1.41 and the heat production 45 per cent above normal. Formation of fat from carbohydrate was found to take place with the loss of only 5 per cent of the energy content of the starch.

Evidence as to the kind of fat formed from carbohydrate is available. Belin (1926) found that fat formed from carbohydrate by mice, rabbits, hens, and ducks had iodine numbers of 73, 60, 79, and 67 respectively. Ellis and Zeller (1930) found that the fat formed by pigs from brewer's

rice and tankage, containing much carbohydrate and less than 1 per cent of fat, was very hard; 97 per cent of it was composed of the glycerides of oleic, palmitic, and stearic acids, and in the mixture a definite relation between the constituents was noted: the amount of stearic acid was half that of palmitic, and the palmitic acid half the oleic.

Food fat. That the fat of the stores may originate from the food fat, and that the food fat may be transferred to the fat stores in the animal organism with very little change is well established from early work (Rosenfeld, 1902). When the percentage of fat in the food is high, the stored fat is very similar to the fat of the food. The statement which appears commonly in the literature, that each species of animal has a characteristic body fat (mutton fat, lard, human fat) appears to be true only when the animal has a considerable variety of food, most of which is not very fatty. Under these circumstances, the animal appears to select from the fat which is delivered into its blood certain fatty acids for combustion and the rest for storage, each species making a different selection according to its peculiar needs. When the amount of fat taken is large, selection is much less effective, and the stored fat then represents the food fat more closely.

This direct transference of food fat to fat depots has given rise to a practical problem of considerable economic importance resulting in much biochemical work (Ellis and co-workers, 1925; 1926a,b; Ellis and Zeller, 1930; Spadola and Ellis, 1936) which has aided greatly in understanding the metabolism of the fats. The "soft pork" problem arose from the fact that when hogs are fattened on food containing a high percentage of low-melting fat or oil, such as the "cake" from the preparation of peanut, soybean, and cottonseed oils, the dressed meat is soft and oily, sometimes to the extent of dripping oil. These are undesirable characteristics and result in price discrimination. It was soon recognized that the undesirable characteristics were the result of the fat of the food. Jackson (1923) has classified the relative softening power of various fats contained in hog feed as follows: Linseed oil has the greatest softening power, then soybean, maize, beechnut, cottonseed, wheat, pea, oat, rice, peanut, barley, rye, and bean oils, in that order. The order is not the same when the concentrates from which the fat has been removed are used as food. Over against the softening properties of the fatty foods may be placed the hardening powers of the grains, which form a high-melting-point fat from the carbohydrate.

Bhattacharya and Hilditch (1931) found that the vegetable fats ingested by animals were utilized by oxidation of part of the linoleic and oleic acids, leaving a residue of palmitic, oleic and linoleic acids more or less adapted to the requirements of the animal. The stearic acid content

of body fats is secured by processes involving saturation of unsaturated fatty acids. Hilditch and Paul (1938) have indicated that there may be arrangements between the saturated and unsaturated members of the same number of carbon atoms, hydrogenation and dehydrogenation—from oleic to stearic acid, for example—which preserve the balance between C_{16} and C_{18} members and yet alter the melting point of the mixture.

Ellis and his co-workers (1925; 1926a,b) found that age in hogs seemed to be a very important factor, *i.e.*, the younger the pig, the softer the fat, the fat of older animals having a higher melting point and a lower iodine number than that of young ones. The rate of fat storage increased with the weight of the pigs, perhaps because as the pigs became fatter, they were less active. Moulton and Trowbridge (1909) found the same fact true for cattle. Parallel with these findings, it was discovered that the proportion of unsaturated fat decreased with age from weanlings up to full-weight pigs. Linoleic acid decreased from 16 to 9 per cent; four-bond acids decreased from 2.5 to 0.7 per cent; volatile acid and oleic acid percentage remained constant. In the fat of the meat (80 to 90 per cent of the whole fat of the animal) four-bond acids were found, but no three-bond acid. The saturated acids consisted mainly of palmitic with less stearic and small amounts of myristic. Linoleic acid in the fat stores followed that in the food very closely, since the pig apparently did not synthesize this acid in fattening. Ellis regarded linoleic as the most important of the food fatty acids from the point of view of softening powers on stored fat. The fact that on a carbohydrate diet (brewer's rice) linoleic acid was 1.9 per cent of the whole stored fat whereas on soybeans it was 30.6 per cent shows the variable percentages of this acid which may be present in stored fat. Iodine absorption and refractive index were found to be the best measures of the firmness of fats, other fat constants showing less correlation. An especially noteworthy fact recorded by Ellis is that linolenic acid (three double bonds), although present in fair amounts in most of the foods, appeared in the stored fat in only two samples out of thirty-six, indicating a preferential combustion of this acid by the animal. A similar failure to find linolenic acid among the fatty acids in the animal body has been reported by practically all recent workers in this field, and is especially significant in view of the importance which has been attached to unsaturation in theories of oxidation of the fatty acids.

According to Ellis, the effect of a change of diet from fat to carbohydrate results in a dilution of the fat already stored with that formed from carbohydrate, which, since it is a hard fat, results in progressive hardening of the stored fat. According to the work of Mendel and Anderson (1926), this can be brought about more quickly by a pre-

liminary period of starvation followed by the carbohydrate diet, since during starvation the liquid acids are apparently removed more rapidly than the solid acids.

Factors affecting the quality of the stored fat were studied by Reed, Anderson and Mendel (1932), who concluded that of all the factors, including food, undernutrition, fasting, muscular activity, ovariectomy, and the administration of thyroxin, the character of the diet and the thyroid hormone represent the only influences that appreciably altered the nature of the depot fat. The fat produced under the influence of thyroxin was more unsaturated—10 to 15 points over the controls. The amount produced was only about one-half that of the controls, although the distribution was the same.

Eckstein (1929) found that:

(1) The main fatty acids formed from carbohydrate are oleic, palmitic, and stearic acids, which is in agreement with the findings of Ellis and Hankins (1925).

(2) Myristic and oleic acids can be transferred from the food to the depots, but butyric cannot.

(3) Arachidonic and linoleic acids and cholesterol appear to be characteristic of the tissue and relatively independent of the diet, although triolein in the food results in small increases in these fatty acids.

(4) Tissues of young rats contain a higher percentage of arachidonic and oleic acids than those of older ones, perhaps because they have less stored fat.

(5) Linolenic acid is not found in the fatty acids of rat tissue, which is in agreement with the findings of Ellis in pigs.

Effect of environmental temperature on nature of stored fat. *In animals.* The nature of the fat stored, as measured by the amount of liquid or unsaturated acid which it contains, appears to depend to a considerable extent on environmental temperature, even in warm-blooded animals. According to the rule enunciated by Terroine and his co-workers, it might be expected that homeotherms, living at a temperature which may be presumed to be adjusted to the optimum for the various bodily processes on a carbohydrate diet, would form and store a body fat of a high degree of saturation. Actually, the fat stored by animals under these conditions was more than half oleic acid, the remainder being made up largely of palmitic and stearic acids (see Ellis and Zeller, 1930). The proportions of fatty acids in the stored fat were found to be oleic:palmitic:stearic :: 4:2:1. The adjustment of the melting point of the stored fat to the body temperature of the animal in its environment, as shown by Henriques and Hansen, is the probable explanation of this apparent

contradiction. To be readily available, the fat stored must remain liquid at the temperature of the storage depots. Fats which become solid would be "frozen assets," not only useless but an encumbrance. On the other hand, a too-liquid fat would be undesirable because of its mobility and also probably because of its oxidizability, a lack of "keeping" quality. Also the more saturated a fat the higher its caloric value, hydrogen being a mighty source of calories.

The relationship between environmental temperature and melting point of stored fat is well brought out by the studies of Henriques and Hansen (1901). They studied the melting point and iodine number of the fat in different locations in the body and found: first, that the fat nearer the outside of the body of animals (subcutaneous fat) had a lower melting point and higher iodine number than that in the interior and therefore warmer regions around the kidney and intestine; secondly, that in pigs with a thick layer of subcutaneous fat, the surface layer had a lower melting point and a higher iodine number than the deeper layers. By temperature measurements at different depths in the thick layer, they found that there was a definite relation between the temperatures and the melting point and iodine number of the fat at these depths. In pigs, the difference in melting point and iodine number was the expression of varying amounts of unsaturated fatty acids. Table 36 is an example of their findings.

Table 36. Melting Points and Iodine Numbers of Fat in Different Locations of the Body of Pigs Fattened on Corn (Henriques and Hansen, 1901).

Fat from	Iodine Number	Solidifying Point (°C.)
Outermost layer of skin fat	72.3	22.8
Second layer of skin fat	70.5	24.1
Third layer of skin fat	65 5	25.7
Innermost layer of skin fat	64.2	25.6
Kidney fat	56.6	28.4
Omentum fat	56 1	29.1

*Typical temperatures**	
1 cm. under the skin	33.7°C.
2 cm. under the skin	34.8°
3 cm. under the skin	37.0°
4 cm. under the skin	39.0°
Rectal temperature	39.9°

* From measurements made with a thermoneedle inserted to different depths.

Dean and Hilditch (1933) confirmed the results of Henriques and Hansen as regards melting point of stored fat in pigs, as just discussed. They criticize the correctness of the statement that warm-blooded animals and tropical plants produce fats of higher melting point than do cold-blooded animals and plants from colder zones, noting many exceptions.

By protecting some members of a group of young pigs from cold and

allowing others to be fully exposed, Henriques and Hansen were able to bring about the deposition of a lower-melting subcutaneous fat in the exposed ones. Henriques and Hansen's experiments confirm in a convincing way the scattered observations found in the literature regarding the relation of melting point of stored fat and temperature of environment. André (1925) gives the following values for the iodine numbers of the fats in different locations in the body of the beef and sheep, showing the striking similarity in different species. In general, the higher the iodine number, the lower the melting point.

	Subcutaneous	Kidney	Marrow	Feet
Beef	80–85	34	40–55	62–95
Sheep	80–85	34	40–55	74

In certain sea animals with a thick layer of fat under the skin, there was the same gradient of melting point, but the iodine numbers increased in the deeper layers. Thus, for the three fatty layers of the skin of the dolphin, the following values were obtained:

	Iodine number	Volatile acids (Reichert-Meissl numbers)
Outer layer	57.6	93.4
Middle layer	89.5	26.5
Inner layer	143.1	14.5

This would seem to contradict the findings on pigs as noted above. However, since the gradient of melting points was the same in the dolphin as in the pig, *i.e.*, the lowest-melting fat on the outside, an explanation was sought and found in the large amount of trivalerin (solidifying point 36°C.) in the fat of this animal. The distribution of the trivalerin is shown by the figures for volatile fatty acids (which would include valeric) quoted above. Although in the dolphin the glyceride which is responsible for the low melting point of the fat is mainly trivalerin, whereas in other animals it is triolein, the same rule holds, *i.e.*, that the melting point of the fat in different parts of the animal body depends on the temperature at that point, and the composition of the fat at any given point is so regulated that the consistency of the fat throughout the organism during life is about the same.

In plants. Very little definite evidence regarding the correlation between environmental temperature and nature of the stored fat in the same plant under different conditions is available. Pigulevskii (1916) made some experiments to test this correlation and came to the conclusion that a cold climate induces in plants the necessity of accumulating a highly unsaturated oil in the seeds.

Studying plants under various climatic conditions, Iwanow (1929) concluded that different species of the same genus under similar conditions formed similar fats. Under different conditions he found that:

(a) On the amount of fatty acids with one double bond, climate and temperature had no effect.

(b) On the fatty acids with three double bonds, climate and temperature had a marked effect—the higher the temperature the lower the quantity of three-bond acids.

(c) On the two-bond acids, the effect was intermediate.

Dean and Hilditch (1933) point out, however, that there are many exceptions to the rule that tropical plants produce fats of a higher melting point than plants in colder regions.

Leathes' conception of the mechanism of the occurrence of the unsaturated acids is that fats are formed in nature by a series of reactions, the end points of which vary according to the temperature at which the reactions occur, the ultimate end point being the large molecular saturated acid (generally stearic acid). At the higher temperatures, the chemical reactions leading to the higher saturated fatty acids reach completion, but at lower temperatures an end point something short of this is reached, resulting in the unsaturated acids.

In studying the effect of environmental temperature on the nature of the stored fatty acids in various organisms, Terroine and co-workers (1927) found that homeotherms, poikilotherms, and plants at high temperatures, as well as molds and bacteria near their upper temperature limit, stored fats of lower iodine number than poikilotherms and plants living at lower temperatures, and molds and bacteria near their lower temperature limits, thus showing the relation between environmental temperatures and nature of stored fat. Pearson and Raper (1927) found that *Aspergillus niger* and *Rhizopus nigricans* produce more saturated fats at the higher temperatures.

Summary. Factors influencing the nature of the stored fat of an animal may then be summed up as follows:

(1) The tendency to form a saturated (hard) fat from protein and carbohydrate, which is modified by:

(2) The necessity of storing a fat which will remain soft, if not liquid, at the temperature of the storage depots. In many herbivorous animals the body fat, after separation from the animal, has a melting point considerably above the body temperature of the animal. In these cases full advantage has apparently been taken of the marked power of supercooling of fat mixtures. There may be a difference of 10 to 15°C. between the melting and solidifying points of these fats. An adaptation of absorbed fat to the body temperature of the animal has been noted during fat absorption. The fat of the chyle may be harder or softer than that of the food which is being absorbed.

(3) The effect of the fat of the food, which, unless metabolized, is laid down in the depots in much the same form as it is ingested. The herbivorous animals, since they live mostly on carbohydrate food, would store a hard fat during most of the year. When fatty seeds and grains mature and are eaten, the relatively highly unsaturated fats which they contain would be transferred to the fat depots and would result in a softer body fat, which provides a natural adaptation to the cold of the winter months by a lowering of the melting point. The carnivora, since they live on the herbivora, would have their fat stores influenced in the same direction.

(4) The effect of selection in the use of fat for energy production. The most marked example of this is linolenic acid which was found by Ellis and associates to be almost eliminated from the food fat before storage. It is curious that this is the acid which both Meyerhof and Hopkins have found useful as an oxygen acceptor and, possibly in the form of peroxides, as an oxygen donator. It is possible that the latter property makes for its selective destruction in the processes of energy metabolism. Perhaps, for the same reason, other still more highly unsaturated fatty acids are found in the stored fat only in traces.

Phospholipids and Sterols

Role as essential cellular constituents

The first notable attempt to link up the phospholipids and sterols with structures and processes in living cells was made by André Mayer and associates, especially Schaeffer and later Terroine. Starting with the conception of living cells as entities in which there are quite constant percentages of essential constituents and therefore quite constant relationships between these constituents, he proceeded to put the lipid constituents to the test of constancy. His attention was early directed to the cell structures known as mitochondria. These structures, according to a review of the literature by Cowdry (1918), are almost universally present in living cells. They are associated with intense protoplasmic activity, for example, cytomorphosis. They are especially abundant in the active stages of life and diminish in senility. Regeneration, compensatory hypertrophy, etc., result in a sharp increase; but decreased activity or decreased oxidation results in a decrease of the mitochondria, often accompanied by an increase of fat.

Mayer, Rathery and Schaeffer (1914) produced evidence to show that the mitochondria undoubtedly contain much lipid. They were soluble in lipid solvents: alcohols, ether, ethyl acetate, chloroform, and carbon tetrachloride; and were insoluble in aldehydes and ketones. They were precipitated by the heavy metals, especially mercury, and by acetone

and formaldehyde. Oxidizing agents rendered them insoluble in the fat solvents, but continued treatment rendered them soluble again. Their behavior in most respects was the same as that of the phospholipids containing unsaturated fatty acids, and the histological technique for the demonstration of mitochondria produced similar effects on these phospholipids. They came to the conclusion then that the mitochondria of the histologists represented, at least in part, the phospholipids of the chemist.

Further evidence that the mitochondria are closely related to the phospholipids is supplied by Bullard (1916) who, finding that the mitochondria of heart muscle were present in twice the amount as in skeletal muscle, correlated this result with that of Erlandsen (1907), who had found that the phospholipid content of heart muscle was also twice that of skeletal muscle. Bullard found that the mitochondria of heart muscle were not decreased by inanition or increased by food, which is in agreement with the findings of Rubow (1905) and Rosenbloom (1913) that the phospholipid content of heart muscle is remarkably constant, even in inanition. On the other hand, analyses of mitochondria by Bensley (1937) do not support the idea that they are largely phospholipid. His figures are: protein 64.67 per cent, lecithin 4.2, cholesterol 2.25, and glycerides 28.88 per cent.

In order to determine just how essential the lipids were in cell life, Mayer proceeded to make a systematic examination of the lipid content and relationships in tissues of various animals under various conditions. Mayer and Schaeffer (1913a) first examined total fatty acids and cholesterol, and found that there was a very considerable constancy in content of these substances in various tissues. Cholesterol especially was found to be constant in percentage in normal tissues (see also Terroine and Weill, 1913), although it increased in long-continued fasts, presumably because some of the tissue was absorbed while the cholesterol was not. In parenchymatous tissues, the variation in total fatty acids and cholesterol was found to be not more than 10 per cent from the mean value, and in muscles 15 to 20 per cent from the mean. The lipid content (lipocytic index) was characteristic of the tissue. The ratio, *cholesterol/total fatty acids* (the lipocytic constant), of different organs was characteristic of the organs. The fact that organs of animals of different species always fell into the same order when arranged according to the value of this ratio therefore suggested correlation with their physiological behavior.

Since cholesterol is always associated with the phospholipids and is in a constant relation to them, it is probably also a normal protoplasmic constituent. In tissues and cells, with the exception of the suprarenals,

it is present mainly in the free form; in blood plasma, it is largely combined as esters of the fatty acids. The relation, *cholesterol/lipid phosphorus*, is believed by Mayer and Schaeffer to be a measure of the ability of a tissue to hold water, the higher the value of the ratio, the greater the water content of the tissue.

Most of the cholesterol in normal tissues is in the free state as shown for typical tissues by the following analyses from Thaysen (1914) (Table 37):

Table 37. Distribution of Free and Bound Cholesterol (Thaysen, 1914). (Per Cent*)

		Free	Bound
Kidney	Dog, lamb and horse	1.31†	0.133
	Human (Windaus)	0.22	0.03
	Rabbit (Ellis and Gardner)	0.16–0.36	0.007–0.11
Adrenals	Horse	2.03	5.5
	Ox	1.66	0.64
Liver	Sheep	0.57	0.24
Heart	Lamb and dog	0.44	0.17

* It is not clear from the author's paper whether the figures are on the basis of dry or moist weight.
† This value is very high as compared with the other kidney values given.

In blood corpuscles, practically all the cholesterol is free. In blood serum about 27 per cent is free and 73 per cent bound (Sperry, 1936).

A discussion of the occurrence of cholesterol esters in tissues as studied by histological methods is given by Kawamura (1927).

Terroine and Weill (1913) confirmed Mayer and Schaeffer's work in finding that the content of total fatty acids and cholesterol (lipocytic index) in liver, kidney, pancreas, lung, spleen, and heart of the dog and rabbit were constant, but that the content in the muscle was variable. They found it impossible to change markedly the total fatty acid and cholesterol content of the parenchymatous organs by either inanition or superalimentation, indicating that these organs do not act as regular storehouses of fat. On the other hand, the content of the muscles did change greatly under these conditions, indicating that the muscle does act as a storehouse of fat. Later, Terroine (1914) added the following: Inanition to the point of death causes an increase of cholesterol in the tissues; this is most marked in the muscle, which may have three times its normal content. He confirmed his earlier findings that the muscle is the most important storehouse of fat, the liver not acting as such in the dog at all, and in birds (pigeons, geese) only in young animals, and then only when the organism is much overnourished.

From their work on the lipocytic constant, Mayer and Schaeffer concluded that a knowledge of the relationships between cholesterol and the phospholipids would give a closer insight into life processes, since the total fatty acids of the lipocytic constant would originate in part

from the fats, which are physiologically inactive. Therefore, they next studied the phospholipids, or rather, the lipid or ether-soluble phosphorus of tissues in relation to cholesterol in tissues (Mayer and Schaeffer, 1913b); this also provided a possible means of distinguishing between the fixed and variable lipid constituents of tissues. They found the ether-soluble phosphorus to be characteristic of the organ or tissue and relatively independent of the species. Inanition or overfeeding (even with food rich in lipid) did not modify greatly the lipid phosphorus content of organs, and therefore the phospholipids did not appear to be an energy reserve. Sometimes the *fatty acid/phosphorus* ratio was less than that of known phospholipids [especially to be noted in the tissues in absolute inanition (Mayer and Schaeffer, 1914a)], but in most cases, especially in muscle and sometimes in liver, it was greater, indicating the presence of fat. They came to the conclusion that the lipid phosphorus content to a considerable extent defines the organ, with the main exception that its amount varies inversely with the size of the animal and therefore directly with its body surface and so with metabolic activity. Thus, the lipid phosphorus of the liver in milligrams of lipid phosphorus per kilo of animal was: for beef, 0.24; man, 0.34; dog, 0.41; rabbit, 0.61; guinea pig, 0.62; and mouse, 0.69. A relation was found between the *cholesterol/lipid phosphorus* ratio and the water content of tissues: the greater the value of this ratio, the greater the water content.

As a result of their finding that the fatty acid content of the tissues of animals fasted to death was insufficient to cover the amount necessary for the ether-soluble phosphorus found, Mayer and Schaeffer (1914a) suggested the possibility of the formation of incomplete phospholipids by removal of part of the fatty acids from the molecule. However, it was pointed out by Le Breton (1921) that ether-soluble phosphorus contains non-phospholipid material.

Cowdry (1918) noted an inverse relation between mitochondria (which, according to Mayer and Schaeffer, are phospholipid in nature) and the neutral fat in tissues. The same idea was expressed by Theis (1928) as a result of his study of the lipids of the liver in different animals under normal and abnormal conditions. Stated briefly, Theis's idea is that there is a relatively constant balance between phospholipid and fat in the liver of normal animals, the relation found being 55 phospholipid to 45 fat. In abnormal conditions, this balance is shifted, the fat being increased and the phospholipid diminished. The following is taken from his data on beef liver:

	Normal liver per cent	Abnormal liver per cent
Total lipid extracted	4.6	4.0
Phospholipid percentage of total lipid	55.1	29.1
Fat percentage of total lipid	44.3	70.0

The phospholipid is both absolutely and relatively lowered in the abnormal liver, and the fat correspondingly increased. In earlier work he found, in animals which had been fed arsenic or phosphorus with resulting fatty degeneration of the liver and heart, that, while the total amount of lipid was practically unchanged, the amount of phospholipid was much reduced; also that insulin caused a reduction of the phospholipid and an increase of the fat in rabbit livers. In the calculations, no account was taken of the fat content; and it is possible, as most workers have found, that on the fat-free basis the phospholipid content may not have been much changed.

Our own work on beef liver (Bloor, 1928) indicates similarly a fairly constant balance between phospholipid and neutral fat in normal beef livers, but with a higher relative content of phospholipid. Thus the phospholipid-to-fat ratio in our samples (with about the same lipid content as those of Theis) was 73 phospholipid to 27 fat. Since the fat fraction as prepared (acetone-soluble fraction) always contains a small amount of phospholipid, the true ratio of phospholipid to fat should then be still higher. With regard to the liver at least, it seems likely that we must be prepared to allow for a considerable variation in the phospholipid-to-fat ratio due mainly to an absolute increase of fat. Recent work on fatty livers (see discussion of lipids of liver, p. 208) indicates that after correction for the fat the phospholipid percentage of liver is very constant. On the other hand, it is equally certain that the lipid percentage in muscle is a very variable quantity depending on the type of muscle and on its metabolic history.

Having obtained evidence of the normal constancy of lipid content in tissues, Mayer and Schaeffer (1914b) attempted to influence the content of lipids of tissues by experimental means. Finding that in hibernants (marmots) the content of parenchymatous tissue in cholesterol, total fatty acids, and lipid phosphorus, varied with the time of year, and that the same appeared to be true with frogs, and assuming that these changes were due to the variations of body temperature, they tried the effects of varying the body temperature (by chilling and overheating) on warm-blooded animals such as rabbits and dogs, species which react quite differently to cold, dogs responding by shivering, rabbits not.

Effects of lowered temperatures were found only in animals which showed a marked reaction to the change of temperature. If the chilling was carried to too low a point (about 25°C. rectal temperature) the animals died without showing any effects on the lipid content of organs; 30°C. was found to be a safe and useful temperature. The effects of lowered temperature were as follows: In the rabbit lung, all lipids increased; in the liver, there was first a decrease and finally an increase in

lipid phosphorus. In the dog, there was a marked change only in the liver, an increase of lipid phosphorus.

Exposure to high temperatures (baths at 39 to 42°C.) produced essentially the same effects: in the rabbit, increases of lipid phosphorus in lungs and liver; in the dog, increases in the liver alone. Injections of diphtheria toxin were also found to cause an increase of lipid phosphorus in the livers of rabbits and guinea pigs.

As to the effect of age and growth on the lipids, Mayer and Schaeffer (1914c) found that the lipid phosphorus percentage was not markedly different at different ages but was higher in young animals (rats).

Terroine and Belin (1927) gave an excellent critical review of the earlier contributions and conceptions of Mayer and Schaeffer and added new experimental support. They pointed out that the earlier methods used, although the best available at the time, were defective in certain respects when reviewed in the light of later work, especially that by Lemeland (1921, 1922, 1923). Thus, after the measurement of cholesterol by Windaus' digitonin precipitation, the considerable remaining unsaponifiable matter was added in with the fatty acids. On the other hand, the fatty acids undergo considerable oxidation in the Kumagawa procedure, and since these oxidized fatty acids are insoluble in petroleum ether they are not included in the total fatty acids. The total fatty acid value is thus increased by the addition of unsaponifiable and decreased by the loss of fatty acids oxidized in the process of separation. Moreover, it has been shown by Le Breton (1921) that ether-soluble phosphorus includes considerably more phosphorus (average, about 20 per cent) than that contained in the phospholipids. Furthermore, the number of organs and tissues worked on was realized to be too small to give results which would be more than a first approximation of the true state of affairs. Mayer and Schaeffer had been impressed by the remarkable constancy of the lipid values for tissues; nevertheless, as Terroine points out, differences exist. The ratio, *total fatty acids/lipid phosphorus*, in the *élément constant* (residual lipid left in the animals which had died of inanition) is very close to that of lecithin, indicating that the fatty constituent of the *élément constant* is lecithin, and also that the lecithin type of phospholipid is the only one present in any considerable amount. The nature of the food was found to be without influence on the fatty acid composition of the *élément constant* over a considerable variety of organisms and tissues. The iodine numbers of the fatty acids of the *élément constant* of muscle, liver, lung, and kidney, are of the same order, although varying considerably among themselves for both hen and rabbit, indicating that the degree of unsaturation of the fatty acids of the

élément constant was probably to be included with the percentage of phospholipids in characterizing a cellular species.

The sum of the results of the French workers gives good support to their conception of variable and constant factors in the lipid content of tissues; the variable constituent is neutral fat, the constant constituents being phospholipid and free cholesterol. Their work indicates also that the constancy is not absolute, but that these constituents can be varied under certain circumstances.

That the degree of unsaturation of the phospholipids is not a constant but may be changed by food was shown by the fact that the phospholipid of hens' eggs may be made to change as the result of diet, and by the work of Sinclair (1932a), who showed that the nature of the unsaturated fatty acids of the tissue phospholipids could be greatly changed in response to food fat, readily in the direction of greater unsaturation, slowly in the direction of a lower degree of unsaturation. Apparently the change took place only in the unsaturated acids, since the percentage of saturated acids in the phospholipids remained constant. Further work by Sinclair (1935c), using a "labeled" fatty acid (elaidic), showed that the tissue phospholipids, especially those of the liver, were readily affected by the food fatty acids and eliminated degree of unsaturation from the constants of the *élément constant.* This work was extended and confirmed by a number of other workers using radioactive phosphorus, and the conclusion is that in some tissues the phospholipid varies promptly in nature with the food fatty acids, while in other tissues the changes are much slower.

Work in this country has shown that variations in the constant factor of tissue lipids (phospholipid and cholesterol) may be quite wide. An extensive study of the phospholipid and cholesterol content of muscle tissue from a variety of animals (Bloor, 1927, 1936; Bloor and Snider, 1934) showed that muscles had no definite phospholipid and cholesterol content but there were (a) differences in content between voluntary, smooth, and heart muscle; (b) differences in different muscles of the same type in the same animal depending on the extent to which the muscle was used; and (c) differences in the same muscle in different species, also depending on the amount of use. Thus in the muscles of the laboratory rabbit, of the voluntary group, the jaw muscles had the highest phospholipid—3.8 per cent—and the cholesterol 0.38 per cent on the dry weight basis; the thigh muscles had phospholipid 1.6 per cent and cholesterol 0.15 per cent. The heart muscle had phospholipid 6.1 per cent and cholesterol 0.57 per cent. The smooth muscle of the stomach (wild rabbit) had phospholipid 2.6 per cent and cholesterol 0.50 per cent. The

pectoralis major in the pigeon, a flying bird, had phospholipid 4.7 per cent and cholesterol 0.25 per cent. In the domestic fowl, which rarely flies, phospholipid was 1.37 per cent and cholesterol 0.17 per cent. The pectoralis muscles of a small bat, which is one of the most expert fliers known, had phospholipid 8.8 per cent and cholesterol 0.6 per cent, almost double the values for the pigeon, which is a good flier. The pectoral muscles of the bat were 6.4 per cent of its body weight, whereas those of the pigeon were 20 per cent. Apparently the smaller size of the bat's muscle was compensated for by the higher phospholipid content. The thigh muscle of a small and very active wild mouse contained phospholipid 6.72 per cent and cholesterol 0.42 per cent, whereas the laboratory white rat's thigh muscle showed phospholipid 3.5 per cent and cholesterol 0.25 per cent. In general, heart muscle had the highest phospholipid content, smooth and voluntary muscle about the same and much lower than the heart. In cholesterol content the smooth muscle generally had the highest content, with heart muscle next and skeletal muscle lowest. Cholesterol content appeared to correlate with automaticity. The following values show the range of cholesterol content: skeletal muscle 0.27 per cent of dry; ventricle of warm-blooded animals 0.55 per cent, cold-blooded animals 0.77 per cent; auricles of turtle and alligator 1.65 per cent; smooth muscle—gastrointestinal tract 0.70 per cent, uterus 1.05 per cent. The phospholipid to cholesterol ratio, *P/C*, was high, 16:1, in voluntary and heart ventricle muscle; low, 3:5, in smooth muscle.

In organs of intermittent activity, such as the corpus luteum and mammary gland, the phospholipid content has been found to increase to a maximum at the time of the maximum activity of the gland (Bloor, Okey, and Corner, 1930; Kaufmann and Raeth, 1927). The cholesterol content often does not increase. When the organ, *e.g.*, the corpus luteum, relapses into inactivity, the phospholipid content falls and the apparent content of cholesterol increases. The increase of cholesterol is often in the form of the esters. In pregnancy, during which the corpus luteum continues active, its phospholipid remains at a high level.

Both phospholipid and cholesterol have been shown to be much higher in malignant than in benign tumors (Yasuda and Bloor, 1932; Bierich and Lang, 1933).

The constant factor in tissue lipids (phospholipid and cholesterol) remains a valuable feature in characterizing a tissue or organ, especially of species with relatively settled habits of activity. It appears, however, to vary with variations in the habitual characteristic activity of the tissue (*i.e.*, in the same working muscle in different animals which do habitually different amounts of muscular work, in organs of intermittent

activity such as the corpus luteum or mammary gland) and with the malignancy of tumors.

Fatty acids in combination in the phospholipids

While the effort of the animal and plant as regards their fat depots is to store a fat as saturated (and therefore as stable) as possible, the opposite seems to be the case regarding the fatty acids of the animal phospholipids. These phospholipids as shown by Sinclair (1932a,b) appear to select the most highly unsaturated fatty acids offered in the food and once selected to retain them.

With regard to the fatty acids in combination in the phospholipids, Terroine and Belin (1927) by assumptions and calculations, but without attempt at direct identification, came to the conclusion that the main unsaturated acid of liver and kidney is linolenic, in the lung linoleic, and in muscle linolenic and arachidonic. Levene and Simms (1921) found that there are equal parts of saturated and unsaturated fatty acids in liver lecithin, and that one of the unsaturated acids is probably arachidonic and the other a C_{18} acid, since it can be converted by reduction into stearic acid. The saturated acids are palmitic and stearic. Theis (1928) found that about one-third of the unsaturated acids of beef liver phospholipid is arachidonic and that very little if any linolenic (three-bond) acid is present. Bloor's (1926) values for arachidonic amount to about 20 per cent of the unsaturated acids, with little if any linolenic. In the phospholipids of other organs and tissues, Bloor found similarly only traces of linolenic acid, arachidonic, linoleic, and oleic acids being the chief unsaturated acids. Ellis and Isbell (1926), in their work on fattening pigs, found that linolenic acid present in the food fat disappeared during the transfer from food to stores. Eckstein (1929), in his work on lipids of rat tissues, makes no mention of linolenic acid. These findings are of peculiar interest because linolenic acid is the unsaturated acid which Meyerhof (1923) and Hopkins (1925) found effective in their oxidation experiments with lecithin.

As regards the ratio between solid and liquid fatty acids in tissue phospholipid, Bloor (1927) found in beef voluntary muscle that about one-third of the fatty acids was solid and two-thirds liquid. In liver (Bloor, 1928), the proportion of solid acids was somewhat higher. Sinclair (1935b) found in the rat that the solid fatty acid fraction constituted a relatively constant proportion of the total fatty acid of the phospholipid, *i.e.*, 30.1 per cent with a standard deviation of only ± 1.6 per cent of the average, quite regardless of the degree of unsaturation of the liquid fatty acid fraction. These findings are in good agreement and indicate

that in the tissue phospholipids there is one molecule of solid acid to two molecules of liquid acid.

The average iodine values of lecithin and cephalin in each organ were so close that a considerable similarity in the two compounds was indicated. When the organs were arranged in the order of the iodine absorption values of the total phospholipid, the brain came highest, liver and kidney next, then lung, with pancreas lowest. The differences in iodine values of the phospholipid fatty acids between the organs were, however, not great (the pancreas was the greatest variant), nor were those of the muscles much different, a fact which points to a considerable basic content of the same phospholipids in all tissues. In view of results by Sinclair (1930) showing that the fatty acids of the phospholipids represent to a considerable extent the fatty acids of the prevailing food, this fact was to be expected. The approximate average iodine number of the intact phospholipids of all tissues was 85, that of the total fatty acid constituents of the phospholipids about 110. Theis (1928), working with beef liver, obtained considerably higher iodine numbers for the phospholipid fatty acids than Bloor did, which may probably be ascribed to normal variations, possibly as the result of the fat of the food (Sinclair, 1930). His values for phospholipid fatty acids averaged 141.

Terroine and Belin (1927) present values for the iodine number of the fatty acids of the *élément constant*, *i.e.*, the lipids (probably phospholipids) remaining in the body after death from starvation of various whole small animals. The values for mice run about 126; for a small bird, 110; and for frogs, 102 to 158, being highest in females because of the accumulation in the ovaries. In larger animals the iodine values for the fatty acids of the organs run remarkably close together (about 140), except for the lung, and appear to be independent of the previous diet. The percentage weights of the *élément constant* are also quite independent of the kind and extent of previous alimentation, thus supporting the findings of Mayer and Schaeffer of an irreducible and essential minimum of lipid in tissues. These findings would seem to require the presence in tissues of a definite percentage of phospholipid, characteristic of the tissue, and containing fatty acids of a definite and relatively constant nature. Our own work is in agreement with this, in that highly unsaturated fatty acids have been found to be characteristic of the phospholipids of the organs. The more recent work of Sinclair (1931) shows that the unsaturation of fatty acids is not a constant factor but varies with the nature of the fat fed, but with a strong tendency for the phospholipids to retain the more unsaturated acids.

Terroine and Belin (1927) and Henriques and Hansen (1903) show that the iodine numbers of the phospholipids of hen eggs may have dif-

ferent values depending on the diet. The differences with food are considerably less than those found in the neutral fat of the eggs, but leave no doubt that the fatty acids of the phospholipids of egg may be changed by food. Terroine and Belin raise the question as to whether all the phospholipid is changeable by this process or whether only a part of it is labile. Shioji (1924) also found that the phospholipid fatty acids of egg yolk could be changed by the fat of the food.

Sinclair (1929), working in this laboratory, has furnished convincing evidence. In an investigation into the mechanism of the absorption of fat in the intestine, he found that during absorption in cats the iodine number of the fatty acids of the phospholipid of the mucous membrane was changed in the direction of that of the fat being absorbed, while the percentage of phospholipid was unchanged. Similar immediate changes in nature of the phospholipid fatty acids were noted only in the liver, those of the other organs and tissues not being affected by a single feeding of fat. By longer feeding, however, it was found possible to change the nature of the fatty acids of the phospholipids of other tissues (kidney, muscle, etc.). Thus the iodine number of the phospholipid of cat tissues was considerably higher on a continuous diet of beef kidney than on a diet of beef muscle. Since the percentages did not change, these results clearly indicate that the fatty acids of some at least of the phospholipids are replaceable, and that therefore the fatty acids of tissue phospholipids are not constant in composition. (The possibility of changing the nature of other protoplasmic constituents, *e.g.*, protein, by the diet, has never been thoroughly investigated by modern methods, although it is generally accepted that such changes are not possible). The evidence in Sinclair's paper goes to show that in certain organs, *e.g.*, the intestinal mucosa and the liver (in later work the kidney also), a portion of the phospholipid content takes part in the absorption and resynthesis of the fatty acids into fat, whereas the other organs and tissues take on new fatty acids only as the wear and tear processes make it necessary for replacement.

Further work by Sinclair (1932a) shows that, although it is readily possible to increase the unsaturation of the fatty acids of phospholipid of the tissues by feeding a fat such as cod liver oil, which has a high iodine number (about 170), it is difficult to decrease it by feeding olive oil (iodine number 86) or coconut oil (iodine number 5 to 10); this makes it appear that the phospholipids of the tissues select the more highly unsaturated fatty acids from the material presented to them, and that these acids are for some reason or other more desirable than the less saturated ones. Having selected and fixed these unsaturated fatty acids in their molecules, they do not readily give them up. This fact raised

a doubt about the participation of the phospholipids as an intermediate stage in the metabolism of fat.

In continuation of his work, Sinclair found in elaidic acid a much more useful "labeled" fatty acid than those of cod liver oil and coconut oil, which he had been using. Elaidic acid is the *trans* stereoisomer of oleic acid, can easily be prepared from it in large amounts, and has the essential advantage that although unsaturated it separates, by reason of its solubility, with the saturated acids in the lead-salt-alcohol separation and so can be readily traced in its progress through the organism. When fed as trielaidin, it is absorbed and apparently as well utilized by the organism as oleic acid. With this material, Sinclair (1935c) was able to corroborate his earlier results as regards entry of food fatty acids into tissue phospholipids. They enter rapidly into the phospholipids of the intestine, liver, and kidney; slowly into those of muscles and tumors; and very slowly into brain and nerve phospholipid. Because of its early entry into and rapid disappearance from intestinal and liver phospholipids, elaidic acid furnished proof that the intestine, liver, and possibly kidney are concerned in the intermediary metabolism of the fatty acids, of which the early stages take place in these organs. Certain other facts noted earlier—the selection of highly unsaturated fatty acids by the phospholipids and the lack of effect of saturated fat in replacing the unsaturated acids of the tissue phospholipids, the lack of proportionality between rate of turnover of phospholipids and intensity of fat metabolism, as well as the constancy in the proportions of saturated and unsaturated fatty acids in the phospholipids regardless of the nature of the diet (Sinclair, 1935a,b)—indicate that the tissue phospholipids may not be concerned in fat metabolism. Sinclair's conclusion regarding this apparent anomaly is that there are at least two classes of phospholipid, differing in composition and function: (a) those containing the more highly unsaturated acids which are especially suitable for functional and structural purposes, such as participation in oxidation processes and the formation of semipermeable cell membranes, etc. and which are protected in the cell from the later stages of metabolism so that they have only the usual tissue wear and tear and replacement; and (b) those which are used for the ordinary metabolic purposes of combustion and energy production, the phospholipid structure rendering the fatty acid more readily transported and metabolized than the fat structure. Sinclair's later observation that the plasma phospholipid, like that of the liver, kidney and intestine, represents closely the absorbed fat is in agreement with these conceptions. The fact that the muscle phospholipid is of the stable rather than of the metabolic class indicates that the muscle, if it uses phospholipid, probably extracts it from the

blood as needed. The observed slow entrance of elaidic acid into muscle phospholipid would represent the replacement of wear and tear.

The fatty acids in the phospholipid of animal tissues originate largely in the food. There is considerable selection as evidenced by the preference for the more highly unsaturated acids and by the fact that the ratio of saturated (solid) acid to unsaturated (liquid) acid is constant. Since some acids are present which are not present in all foods (arachidonic acid it is probable that there is some synthesis.

Relation of lipid content to physiological activity

Having noted the great differences between the phospholipid and cholesterol content of the same muscle in animals of widely different activity (p. 250 ff.), an attempt was made to produce these differences experimentally. Matched pairs of rats of about 150 grams weight were selected (Bloor, 1937). One of each pair was confined in a small cage, the other allowed to exercise at will in an 18-inch exercising wheel made according to Slonaker (1908). After a preliminary learning period, most of the rats ran freely, averaging the equivalent of about 5 miles per day. After one to two months, they were sacrificed and their muscles analyzed and compared with similar analyses of the controls. A similar comparative study was made of flying and confined pigeons. In the rats, the effect of exercise showed itself in increased phospholipid in all muscles except in the diaphragm (which was greatly hypertrophied) and notably in those muscles concerned with running: heart, front leg, gastrocnemius, and back. Changes in cholesterol in general followed those of phospholipid, but to a less extent. Hypertrophy was general in these muscles (as shown by change in weight relative to body weight). In the pigeons, the effects of exercise were increases of phospholipid of 18-25 per cent in the flight muscles without hypertrophy, together with decreases of cholesterol. The value of the findings was lessened by the fact that individual differences between animals were so great that, according to the usual rules of statistical treatment, the results by themselves were not significant. On the other hand, the changes were all positive and in the same direction as the natural differences found. For this reason, it is believed that the results are significant and represent one type of adaptation to increased muscular activity.

Another well recognized type of adaptation of muscle to increased work is hypertrophy. It was found in the work reported above that the only muscle which hypertrophied greatly did not show increases in phospholipid content. Similar lack of change in phospholipid content in hypertrophy was found for kidney and heart by Ludewig and Chanutin (1936). In unpublished work from this laboratory, we have been able

to show that although uterus muscle in the rabbit increases to about eight times its resting weight during pregnancy, the phospholipid and cholesterol content do not change. Similar results were obtained with kidneys made hypertrophic by the high-protein diet of MacKay and associates (1928). It seems clear, therefore, that when a tissue hypertrophies as a result of increased work its lipid content need not change. In atrophy, the muscle generally decreases in phospholipid and increases in cholesterol.

Exercise of frog muscle tetanically until fatigued, either in hydrogen or oxygen with recovery, did not change the amount or iodine number of muscle fatty acids (Winfield, 1915; Buchwald and Cori, 1931).

Embden and Lawaczeck (1923) found that the greater the endurance of a muscle, the higher its cholesterol and rest phosphorus, which Sorg (1929) has shown to be phospholipid.

The relation between the lipid content of muscles and their fatigability has been examined by Sorg (1929). He found that slowly fatiguing muscle had a higher cholesterol value than rapidly fatiguing muscle, and that the heart had the highest cholesterol content of all the muscles. He found also a parallelism between phospholipid content and fatigability. The heart has the highest phospholipid of all the muscles and is very difficult to fatigue.

Boyd (1937) found no significant relation between hypertrophy of the heart and oviduct of frogs and their content of phospholipid and cholesterol.

Caminade, Mayer and Vallée (1927) found that while moderate physiological activity had little effect, maximum nervous activity increased the phospholipid content noticeably.

Lipids of Plants

A great deal is known about the neutral fat of plants, especially of plant seeds, but since the point of view is economic and commercial rather than physiologic, much of the information need not be discussed here. The reader is referred to numerous texts on fats and oils (*e.g.*, Jamieson, 1932). The remaining material on plant lipids is discussed according to whether it deals mainly with fat, phospholipid, or unsaponifiable material.

Fat

Starches. Fatty acids found in natural combination with carbohydrate are well known (Taylor and Nelson, 1920), and may be demonstrated and determined after destructive hydrolysis of the compound. Lehrman (1930) has found the following percentages of acids in the mixture obtained from wheat starch: palmitic, 35 per cent; oleic, 41 per

cent; and linoleic, 24 per cent. In corn starch, Taylor and Lehrman (1926) found the fatty acids entirely combined in the α-amylose or insoluble portion. The acids were the same as noted for rice and wheat starch. They are liberated only after long hydrolysis with acid. In banana starch, Lehrman and Kabat (1937) found 0.2 per cent of fatty acid consisting of palmitic, oleic, linoleic, and linolenic acids with a small amount of phytosterol. As pointed out by Evans and Lepkovsky (1932), the small amount of fatty acid, especially linoleic, combined in the starches is of physiological importance in the so-called fat-free diets which contain starch, as a protective agent against the fat deficiency disease.

Algae. Takahashi, Shirahama and Tasi (1933) found that the differences in the fat content of algae by colors were brown > green > red. The iodine number of the fats were brown > red > green. The fatty acids found were capric, caproic, caprylic, myristic, oleic, and linolenic.

Plankton. Plankton oils have been studied by Collin and associates (1934). The zoöplankton oils were found to resemble those of cod liver oil. The unsaponifiable fraction contained cholesterol, a hydrocarbon (squalene or a similar substance), cetyl, and eicosyl alcohols.

Phospholipid

Studies have been made of the phospholipids of beans (*Vicia Faba*) by Magistris and Schäfer (1929). Many of these substances may be dialyzed out of the plant tissues into a mixture of ethyl and capryl alcohol in water, and when so separated are found to consist of phospholipid in combination with a carbohydrate component. These authors regard ordinary phospholipid as a denaturization product of this naturally occurring phosphatide.

Winterstein and Hiestand (1908) found a substantial carbohydrate content (up to 25 per cent) in soybean phospholipid. The carbohydrate was a polysaccharide of glucose, galactose, and pentose or methyl pentose.

Levene and Rolf (1924) found in the lecithin of the soybean stearic, palmitic, oleic, linoleic, and linolenic acids, with a small amount of acids insoluble in petroleum ether. As compared with animal lecithins, there was a relatively low proportion of saturated acids, no carbon chains longer than C_{18}, and a notable amount of linolenic acid, which is rare in animal lecithins. The animal lecithins are characterized by the presence of C_{20} to C_{24} acids. Yokoyama and Suzuki (1931) examined the different lecithins of soybean and found in the α series palmito-linoleic, oleo-linoleic, dioleic, and dilinoleic lecithins. Suzuki and Nishimoto (1930) found that the fatty acids of a cephalin of soybean consisted of 50 per cent stearic acid and 50 per cent of a mixture of equal parts of

linoleic and linolenic acids. This particular cephalin is interesting because it is a β-cephalin. Horvath (1935) found that soybean may contain up to 3 per cent of the weight of the seed as phospholipid, which is the same content as that of whole hen's egg. The soybean lecithin gave low values for phosphorus, which the author thought was due to its content of carbohydrate, a characteristic contaminant of plant phospholipids, as noted above.

In corn pollen, Anderson (1923) found a phospholipid containing 4.09 per cent phosphorus, probably a typical lecithin. Heé and Bayle (1932) found that the phospholipid of the cotyledons of *Lupinus albus* largely disappeared during germination.

In rape seed, the crude phospholipids were found associated with a polysaccharide. The purified product was 60 per cent insoluble in boiling ethyl alcohol (cephalin fraction), 20 per cent soluble in boiling but insoluble in cold alcohol, and 20 per cent soluble in cold alcohol (lecithin fraction). The phosphorus and nitrogen contents of the three fractions were 3.7, 1.42; 3.14, 1.54; 3.14, 1.42, respectively. The phosphorus-to-nitrogen ratio was 1 in each case (Rewald, 1937).

Rewald (1929) found, in potatoes and beets, phospholipid up to about 0.5 per cent of the dry weight. In fruits, the following phospholipid values were found: cherries, 0.03 per cent; apples 0.09 per cent; plums 0.066 (skin); apple 0.011 (skin). Crude fat values were: in plum flesh 0.37 per cent, in pear 0.11, in cherry skin 1.57, in pear skin 0.54.

In cabbage leaves, Chibnall and Channon (1929) found that lecithin and cephalin were absent and that the main phospholipid was calcium phosphatidate (a lecithin or cephalin with the choline or colamine replaced by calcium). In the runner bean, Jordan and Chibnall (1933) found lecithin and cephalin in progressively smaller amounts from the cotyledons to the pinnate leaves. The cotyledons and embryo axes contained a small amount of magnesium phosphatidate which on germination increased rapidly, the magnesium being slowly replaced by calcium. The calcium phosphatidate was the chief phospholipid of the mature prophylls and pinnate leaves. The seed phospholipids were believed to serve as food reserves, while those of the leaves were integral parts of the protoplasm. In starvation experiments, the waxes and unsaponifiable remained unchanged and were therefore regarded as end products of metabolism.

In cocksfoot grass, Smith and Chibnall (1932) found lecithin and cephalin, in addition to phosphatidate. The fatty acids present were linoleic, linolenic, and saturated acids, but no oleic. The ratio of saturated to unsaturated acids was less than unity.

The yield of acetone-insoluble material from barley was 0.16 per

cent, wheat 0.17, oats 0.14 (Diemair, Bleyer and Schmidt, 1937). It consisted of monoamino phospholipids of the lecithin-cephalin type, was free of carbohydrate but had a low phosphorus content. In barley and wheat, the α form was the one present, in oats the β form. In barley, palmitic and stearic were the main solid acids; in the others, myristic acid was also present. Choline was the only base found. In wheat germ, phosphatidic acid, lecithin, and cephalin were found in the proportion of 4:4:1 (Channon and Foster, 1934).

The food fat of a large tree (110-year old beech) was studied by Gäumann (1935). In the whole tree about 6.1 kg. of fat were found, chiefly in the wood. This reserve was used up in the spring before the tree leaved out. Very little useful material was lost by the falling of the leaves, most of the food material having been withdrawn before the leaf left the tree.

Unsaponifiable

In the endosperm of corn, Anderson (1924) found two fractions of phytosterol differing in properties and condition. The endosperm of corn also contains some free phytosterol: melting point 137-137.5°C., specific rotation −32.23°, acetyl derivative melting point 127°C. After saponification the unsaponifiable matter was separated into the following three parts:

(1) The optically active dihydrositosterol, $C_{27}H_{47}OH.H_2O$, melting point 138-139°C.; the dried preparation melting between 140-141°C., $[\alpha]^{20}_{D} = 25°$; the acetyl derivative melting point being about 138°C., $[\alpha]^{20}_{D} = 14.41°$.

(2) Rather large quantities of the ordinary sitosterol associated with dihydrositosterol in the endosperm and bran of corn.

(3) A brownish yellow oily substance that was not examined.

Dihydrositosterol crystallizes in the same form as sitosterol but the crystals are larger and denser. It does not give the Liebermann-Burchard reaction, and the Whitby reactions are atypical. It does not absorb bromine.

Anderson (1923) found in the lipids of corn pollen:

(1) Phytosterol palmitate which melted at 88-88.5°C. On saponification this substance yielded: (a) palmitic acid melting at 62.5°C., (b) one fraction of phytosterol melting at 122°C., and (c) another fraction of phytosterol which melted at 136.5°C. The acetyl derivative of the latter melted at 101°C.

(2) A saturated alcohol, $C_{30}H_{62}O$, which melted at 136°C.

(3) That the fat extracted from corn pollen with absolute alcohol contained 25 per cent of unsaponifiable matter consisting of a mixture

of phytosterols. The melting points of these phytosterol fractions ranged from 121 to 154°C. The acetyl derivative of the latter melted at 134°C.

(4) That the ether extract contained 14 per cent of the saturated hydrocarbon, $C_{29}H_{60}$, melting point 63°C., and 4.4 per cent of phytosterol melting at 125-126°C.

The phytosterol preparations isolated from corn pollen differed from ordinary phytosterol in several particulars: (a) They were all free from water of crystallization. (b) They were optically inactive. (c) The melting points of these phytosterols as well as of their acetyl derivatives all differed from those of ordinary phytosterol.

In various common oils, Anderson and Moore (1923) found sterols as follows:

(1) Corn oil contains a relatively high percentage of unsaponifiable matter, amounting, in the crude oil, to 2.01 per cent and in the refined edible oil to 1.68 per cent. This unsaponifiable matter consists largely of phytosterol. Its melting point is 137.5°C., and the optical rotation of the dry phytosterol is 34.38°. The acetate melts at 127°C. The phytosterol of corn oil does not contain any stigmasterol.

(2) Cottonseed oil contains at least two phytosterols which differ in melting points and probably in optical rotation. Two fractions of phytosterol were separated by fractional crystallization: (a) Melting point 138-139°C.; $[\alpha]^{20}_{D} = 34.19°$; the acetate melted at 124°C. (b) Melting point 134-135°C.; $[\alpha]^{20}_{D} = 33.61°$; the acetate melted at 119°C.

(3) Linseed oil contains at least two phytosterols which differ in melting point and optical rotation. As in the case of cottonseed oil, two fractions of phytosterol were separated by fractional crystallization: (a) Melting point 138°C.; $[\alpha]^{20}_{D} = 34.22°$; the acetate melted at 129-130°C. (b) Melting point, not sharp, 134°C.; $[\alpha]^{20}_{D} = 31.16°$; the acetate melted at 124°C.

Mixtures of phytosterols such as are obtained from cottonseed oil and linseed oil are so nearly alike in solubility in the usual solvents that it is practically impossible to effect a complete separation by simple fractional crystallization.

Later Anderson, Nabenhauer and Shriner (1927) reported that:

(1) The saturated sterol, dihydrositosterol ($C_{27}H_{47}OH$), is apparently rather widely distributed in plant fats.

(2) It occurs in association with unsaturated sterols, not only in the endosperm and bran of corn and wheat, but also in the oils obtained from the germ of these grains.

(3) Appreciable quantities of the substance have been isolated from corn gluten, corn bran, wheat bran, rice bran, corn oil, and wheat germ oil.

(4) The dihydrositosterol preparations that have been obtained from different sources show slight variations in physical properties. The melting points vary from 141-2 to 145-6°C., and the specific optical rotations vary from about +23 to +25°. The acetyl derivatives vary in melting points from 137 to 141°C. and in optical rotation from about +13° to +14°. Whether these variations in properties depend upon the degree of purity, or whether they are due to the presence of isomeric saturated sterols cannot be determined from the present data.

As regards cholesterol, Anderson (1927a) showed that cholesterol preparations obtained from different sources have slight differences in physical properties. When apparently pure cholesterol acetate is fractionally recrystallized from ethyl alcohol, it is possible to separate a bottom fraction that possesses a much lower melting point and a lower optical rotation than the top fraction.

In green leaves (spinach and cabbage), Collison and Smedley-MacLean (1931) isolated from the unsaponifiable substance: carotene ($C_{27}H_{46}O$), a sterol, ceryl alcohol ($C_{27}H_{54}O$), and a hydrocarbon ($C_{31}H_{64}$).

Chibnall and associates (1931b) found *n*-hexacosanol in cocksfoot grass; *n*-triacontonol, m.p. 86°C., in lucerne (1933a) along with paraffins of the molecular weight of triacontane (m.p. 66°C.). In wheat, they (1933b) found *n*-octacosanol and a complex mixture of hydrocarbons.

The waxy coat of the skin of the Ben Davis apple has been investigated by a number of workers (Sando and associates, 1923, 1932; Piper and associates, 1931) who found *n*-nonacosane, m.p. 284-5°C., a crystalline alcohol. Chibnall and associates (1931a) found substantially the same.

MacLachlan (1936b) studied the lipids of the soybean during germination. The total fat of the cotyledons diminished markedly as germination proceeded, the decrease being more marked in the light than in the dark. The roots, stems, and leaves built up their fat equally well in the dark and in the light, and the fatty acids so formed were considerably more saturated than those of the cotyledons. There was a marked increase in the sterol content of the beans, which occurred chiefly in the roots, stems, and leaves of the young plants. Esterification of the sterol occurred mostly in the cotyledons (the ester is regarded as the inactive or storage form of the sterol). MacLachlan concluded that there was a close relation between the metabolism of the sterol and the utilization of fat.

Beumer (1933) found that sterol formation in plants proceeded actively during germination and growth.

In tobacco seeds, the total lipid was found to be 35.8 per cent, containing 0.15 per cent of sitosterol and 0.07 per cent of phospholipid. The

fatty acids were: linoleic 56.3, oleic 28, palmitic 9.8, and stearic 5.9 per cent (Salisbury, 1937).

The growing tips of young pines (*Pinus caribaea*) yielded C_6-C_9 paraffins; a trace of α-pinene; melissic acid ($C_{30}H_{60}O_2$) and melissyl alcohol; *n*-nonacoson-10-ol; sitosterol ($C_{29}H_{50}O$) and a sitosterolin; and palmitic, behenic (m.p. 78°C.), oleic, linoleic, and abietic ($C_{20}H_{30}O_2$) acids (Hall and Gisvold, 1936).

In the rice embryo, Tanaka (1933) found mylicyl alcohol, dihydrositosterol, stigmasterol, and sitosterol.

Lipids of Microörganisms

The available work on the fatty substances of microörganisms is small in amount and may be criticized in some cases on the score of faulty technique. Ordinarily the results are those obtained by fat-solvent extraction (mainly ether) of the dried bacteria, which, in the light of our present knowledge is inadequate to remove all the lipid. Even boiling alcohol, probably the best known lipid solvent, has been shown by Smedley-MacLean (1922) to leave behind considerable of the fatty material of yeast which could be liberated only after hydrolysis with acids. The same has been shown by Terroine and Lobstein (1923) for tubercle bacilli, as much as 5 per cent of the lipid being left behind after exhaustive extraction in a Kumagawa-Suto apparatus. Some of this unextractable residue is probably combined with either the carbohydrate or protein as it is in starch. The earlier results on the ether-extractable material of various bacteria have been reviewed by Buchanan and Fulmer (1928). For many of the more recent facts regarding the lipids of bacteria, we are indebted to Anderson and his associates.

With the exception of the sterols, which appear to be absent from many bacteria, practically all the known types of lipid, or some modification of them, are found in the microörganisms, which was perhaps to be expected since their metabolism is not very different from that of other cells. The fatty acids found in lipid combination are those found in the higher organisms. Palmitic, stearic, and oleic acids are generally present and comprise the most important constituents. Lower fatty acids (butyric and caproic) are reported by Goris and Liot (1921). In general, the more unsaturated higher fatty acids are less well represented than in animal cells, although Smedley-MacLean and Thomas (1920) found linoleic acid in yeast.

In addition to forming and storing the lipids in their cell bodies and as a corollary to these processes, many microörganisms can bring about various changes in lipids in their culture media. Bacteria may induce oxidative rancidity (lipase, peroxidase), hydrolysis with formation of free

fatty acids (lipase), tallowiness (oxidizers) in beef and mutton fat. Fat-soluble pigments of various microörganisms cause pink fats and purple "stamping ink" discolorations by oxidation-reduction mechanisms. Pure fats, free from moisture, apparently do not support the growth of microörganisms (Jensen, 1937). Vickery (1936) tested the lipolytic activity of several strains of *Achromobacter* and *Pseudomonas* and also of asporogenous yeasts on a synthetic medium containing 80 per cent beef fat in water-in-oil emulsion. All the yeasts and *Pseudomonas* were active as hydrolyzers, but only one strain of *Achromobacter* had this power to a slight degree. The contention that the level of free acidity of beef fat could be used as an index of spoilage could not be confirmed as of general application.

As already noted, many if not most bacteria contain no cholesterol or other sterols of like nature, but Hecht (1935) found that substances giving the ordinary cholesterol reactions were present in a number of bacteria, including tubercle and timothy grass bacteria. Sifferd and Anderson (1936) found in the unsaponifiable part of the acetone-soluble lipids of *Azobacter chroococcum* a sterol giving a precipitate with digitonin but different from other plant sterols and more like ergosterol.

A comparison of the development of bacilli grown on potato media containing (a) 0.07 per cent cholesterol, (b) nothing additional, (c) 0.1 per cent lecithin, and (d) 0.07 per cent cholesterol + 0.1 per cent lecithin showed that the rate of growth increased in this order; the order of toxicity of such cultures for white mice was (c) (b) (d) (a) and for guinea pigs (d) (c) (b) (a).

Bacteria

The lipids occurring in acidfast bacteria are built on quite different plans from those found in plants and animals and their composition is more complex (Anderson, 1941). The acetone-soluble fats are mixtures of neutral fat and large amounts of free fatty acids. In addition to the ordinary fatty acids, there are new and specific fatty acids. Two of these, tuberculostearic (10 methyl stearic) and phthioic ($C_{26}H_{52}O_2$ of unknown structure) have been obtained in pure form. The fats of human, avian, or bovine tubercle bacilli are not glycerides but probably esters of carbohydrates, one of which, trehalose, has been separated from human bacilli. The leprosy bacillus also contains trehalose, in spite of the fact that this organism as well as the others was grown on a glycerol medium. The phospholipids from acidfast bacteria resemble ordinary phospholipids in solubility but differ in most other respects. They contain 2.6-3.5 per cent of phosphorus and very small amounts of nitrogen, part of which is ammonia. The phosphorus is in part combined as glycero-

phosphate, in part as phosphorylated polysaccharide. Mannose, inositol and a hexose which yields glucosazone are also found in phosphoric combination. The fatty acids include saturated, high-molecular, branched-chain fatty acids which are liquid at ordinary temperatures. The waxes are quantitatively the most important of the ether-soluble constituents. They are esters of optically active hydroxy acids with carbohydrates and differ with each strain of bacteria. The polysaccharides found in these waxes include trehalose and, in the case of human tubercle bacillus, a specific polysaccharide not found in other acidfast bacilli. The wax from the human bacilli contains a polysaccharide which yields on hydrolysis mannose, arabinose and galactose. The principal fatty acid is mycolic ($C_{88}H_{176}O_4$) containing a hydroxyl group and a methoxyl group.

The lipid content of bacteria has been found to vary greatly with the nature of the medium on which they are grown. Cramer (1893) and Lyons (1897) found that the pneumobacillus more than doubled its fat content when sugar was added to the culture medium. Frouin (1921) found that glycerol in the medium was especially effective in increasing the fat content of tubercle bacilli. Similar results have been reported by Long and Campbell (1922) for various acidfast bacilli, the glycerol being particularly effective in increasing the amount of wax. Larson and Larson (1922) sought to establish the general law for all bacteria that carbohydrates and glycerol are converted into fats or fat-like substances only when they are not fermented by the organisms. They found that the fat content of the tubercle bacillus bore no relation to the virulence of the organisms but was determined by the action of the bacteria on the glycerol in the medium. The acidfast staining properties of the tubercle bacillus were due not to its high fat content but probably to the character of the fats or other substances present. Haag (1928) found that the ease of decomposition of fat by bacteria depended on the degree of unsaturation and also on the steric arrangement, oleic and erucic being more easily decomposed than isomeric elaidic and brassidic acids.

Much of the available information on the lipids of bacteria has been obtained in the effort to find out the reason for acidfastness in staining reactions. In general, only lipid-rich bacteria are acidfast. The lipid content of acidfast bacilli was determined by Long and Campbell (1922). Tubercle bacilli of human, bovine, avian, frog, and turtle types; leprosy, smegma, dung, and grass bacilli, *B. subtilis*; and a strain of *Staphylococcus aureus* were among those examined. The bacilli of the acidfast group were found rich in lipid as compared with non-acidfast bacilli. In most cases, 20 to 35 per cent of the dry weight could be extracted with petroleum ether after dehydration with hot alcohol. Quantitatively, there was a great difference in the lipid extracts. The bacilli fell into three

groups in respect to the proportion of the total lipid which was present as wax (unsaponifiable lipid). This wax consisted of a difficultly hydrolyzable combination of fatty acid with an alcohol of high molecular weight. The lipid of the human and bovine type tubercle bacilli was 60 to 77 per cent wax. That of avian type tubercle bacilli and the leprosy bacilli of frogs and turtles ranged between 27 and 36 per cent wax (the higher value being for avian bacilli). The wax content of smegma, dung, and grass bacilli of various types ranged from 4 to 10 per cent of the total lipid. A closely similar grouping of the same series was made on the basis of nutrition, and a similar grouping with respect to virulence was obvious, although not stressed without further study. Sufficient data were secured to show that the microörganisms listed formed wax somewhat in proportion to their glycerophilism, and therefore glycerol may be looked upon as a wax progenitor.

Chargaff (1931) compared non-pathogenic acidfast bacteria (turtle tubercle and smegma bacteria) with human and bovine tubercle bacteria and found that the former were characterized by their lower wax and higher methyl acetate-soluble fat content. The fat of the turtle bacilli, like that examined by Anderson, consisted partly of true glycerides and partly of a fatty acid compound with a polysaccharide. The unsaponifiable portion gave none of the usual reactions for sterols. The phosphatide contained 3.16 per cent phosphorus and 0.39 per cent nitrogen.

Chargaff also examined the diphtheria bacillus and gave figures for the data found. A striking difference between the diphtheria bacillus and the acidfast tubercle bacteria was the low wax and total lipid content of the former. The lipid of the diphtheria bacillus (Chargaff, 1933) consisted mainly of free fatty acids. The total lipid was 4 per cent, and phospholipid 0.4 per cent. From the ester portion no glycerol was obtained on hydrolysis, but an amorphous polysaccharide soluble in acetone. The unsaponifiable portion contained no sterols but a highly unsaturated substance (iodine number 140). The solid fatty acid was palmitic, the chief liquid acid palmitoleic. The higher unsaturated fatty acids contained diphtheric acid ($C_{35}H_{68}O_2$), m.p. 35-6°C. The phospholipid yielded aldohexose on hydrolysis. The mixed fatty acid had an iodine number of 93.

Lactobacillus acidolphilus was found (Crowder and Anderson, 1934) to contain about 7 per cent of ether-soluble lipids, consisting of 28 per cent of free fatty acids (of which 3.4 per cent was dihydroxystearic), 35.2 per cent neutral fat, and 32 per cent phospholipid. The neutral fat yielded on saponification 81.5 per cent fatty acids, 12.5 per cent glycerol, and 6.7 per cent unsaponifiable, of which the crystalline portion was cholesterol. The total fatty acids consisted of 57.8 per cent solid satu-

rated acids (lauric, myristic, palmitic, and stearic) and 36.9 per cent unsaturated acids, entirely oleic. The phospholipid yielded 55 per cent fatty acid, glycerophosphoric acid, choline, and over 20 per cent of a polysaccharide. Solid fatty acids were the ordinary ones plus a tetracosanic acid; the liquid acids reduced to palmitic and stearic. The polysaccharide yielded on hydrolysis *d*-galactose, and apparently glucose and fructose. When hydrolyzed with dilute alcoholic potash, a polysaccharide was obtained containing 9.5 per cent phosphorus. When this was removed with ammonia under pressure, a crystalline, non-reducing polysaccharide was obtained, showing mutarotation, with a final rotation of +72°. The choline isolated was only a fraction of the total nitrogen, and no aminoethyl alcohol was found. This phospholipid is apparently a mixture of the glycerol-choline compounds common to most living things, and the polysaccharide compounds characteristic of bacteria, especially of the acidfast bacteria.

Timothy bacillus. Stephenson and Whetham (1922) became interested in the timothy bacillus because it had a high fat content and was related to the tubercle bacillus. It was grown on a synthetic medium containing potassium hydrogen phosphate, ammonium diphosphate, magnesium sulfate, a trace of sodium chloride, a source of carbon not more complex than glucose, and an excess of calcium carbonate to maintain the pH at approximately 8.0. When 1 per cent of glucose and 1 per cent of acetic acid (as sodium acetate) were used as the source of carbon the growth of the bacillus, as measured by the synthesized protein and lipids, attained its maximum at the point when glucose and acetate disappeared from the medium. Thereafter the bacillus utilized its own lipids and these rapidly decreased. At first the fat fraction was used more rapidly than the phosphatide fraction, until an equilibrium was established and the phosphatide was maintained at a higher value. Apparently the fat was stored material while the phosphatide composed some unit essential to the chemical structure of the cell. When lactic acid in the form of its sodium salt was used as the source of carbon, the growth was very similar to that on glucose alone, as might have been expected from its relationship to glucose; the lactic acid was completely utilized and the formation of protein and fat resembled that occurring on a glucose medium. When acetate in the form of its sodium salt was used as the source of carbon, growth was negligible, and the acetate was not attacked by the bacillus. When, however, a mixture of acetic and lactic acid (sodium acetate and calcium lactate) was used as the source of carbon, the presence of the lactic acid enabled the bacillus to utilize the acetic acid. The presence of glucose likewise enabled the bacillus to utilize the acetic acid. However, the acetic acid which was used in the

presence of either lactic acid or glucose did not increase the general growth of the organism, but did increase the amount of lipids formed; this was a specific effect of the acid and was not caused merely by a greater concentration of carbonaceous food. When either propionic acid or normal butyric acid was used as the source of carbon, the bacillus grew without the addition of other compounds of carbon and synthesized both proteins and lipids.

Pangborn and co-workers (1931; 1932) separated and studied the lipids of the timothy bacillus. The "fat" was not a glyceride but contained a carbohydrate or polyhydric alcohol. The "unsaponifiable" was a dark oil with iodine number of 126, and gave no sterol reactions. The fatty acids of the fat consisted mainly of palmitic acid and a liquid saturated acid, probably tuberculo-stearic acid. The unsaturated acid appeared to be an unsaturated derivative of palmitic acid. The phospholipid contained 60 per cent of fatty acid, consisting of palmitic, phthioic and unsaturated derivatives of palmitic and stearic acids. The water-soluble constituents were glycerophosphoric acid, inosite and mannose; only a trace of nitrogen was found.

Tubercle bacillus. For obvious reasons much attention has been devoted to the lipid content of the tubercle bacillus. Koganei (1922) found that the fatty substances of tubercle bacilli contain phrenosin, kerasin, sphingomyelin and cephalin, besides neutral fat and fatty acids. The first four substances had not previously been reported as constituents. He also studied the acidfast property of each isolated constituent to find out which was responsible for this characteristic property of the bacilli. Small quantities were fixed on slides with egg albumin and stained with Ziehl-Neelson solution for 5 minutes with slight warming. The decolorizing was accomplished with a 3 per cent alcohol solution of hydrochloric acid. Only the portion soluble in ether but insoluble in alcohol and acetone was found to possess this property. This portion represented a mixture of wax and cephalin. When the material was saponified with sodium ethylate for 20 hours and the unsaponifiable substance emulsified with boiling water for several hours, a colloidal solution formed from which the waxy matter could be separated because it floated to the surface. This waxy matter, which lacked phosphorus and seemed to be an aliphatic alcohol, did not display the acidproof property. On the contrary, the colloidal solution, when concentrated and dried, did show acid resistance. To establish more definitely that the cephalin was the substance responsible for the acidproof property, experiments were also made with cephalin isolated from ox brains. These results, as well as the fact that cetin and beeswax failed to give the reaction, corroborated his conclusion that the acid resistance was due to the cephalin.

Terroine and Lobstein (1923) found that, under identical conditions of culture, tubercle bacilli of bovine origin contained much more lipid than those of human origin. In both strains the lipid content diminished to 60 per cent of its value if glucose was substituted for glycerol, the carbon content and other constituents remaining identical. The unsaponifiable portion, treated with an alcohol solution of digitonin, yielded 0.16 to 0.45 per cent of a cholesterol-like substance giving marked Salkowski and Liebermann-Burchard reactions. Its ratio to total lipids was very constant for bacilli of the same origin. It was higher (1.8 per cent) for bovine than for human (1.3 per cent). Where glucose or glycerol was used, the fatty acids formed had approximately the same melting point, but with glycerol the iodine number was a little higher. The protein content was higher with glucose than with glycerol (11.6 and 8 per cent) and was thus in inverse relation to the lipid content.

Frouin (1921) noted that the amount of material recovered from *B. tuberculosis* by fat solvents varied according to different investigators from 4 to 46 per cent. Various solvents under diverse conditions have been employed, but the type of bacillus, the age of culture, and the composition of the medium upon which it was cultivated have usually been disregarded. He cultivated human, bovine, and avian strains on a medium composed of dipotassium phosphate, magnesium sulfate, sodium citrate, asparagine, and glycerol. Cultures grown for a month upon such media gave the percentages of lipid material shown in Table 38:

Table 38. Lipid Material in Human, Bovine, and Avian Tubercle Bacilli on Different Media (Frouin, 1921).

B Tuberculosis	Asparagine and Glycerol (%)	Asparagine, Glycerol and Glucose (%)	Asparagine, Manitol and Glucose (%)
Bovine strains	22.95	23.10	12.99
Bovine strains	45.51	43.85	12.99
Bovine strains	42 86	45.51	8.55
Human strain	19.59	21.32	6.75
Avian strain	40.10	39.23	14.22

The effect of the glycerol is noteworthy and the comparison of the fat and wax content of human and bovine bacilli grown under identical conditions suggests that this chemical factor may be associated with the virulence of the strains for laboratory animals.

Long (1923) found that bacilli of the acidfast group which had been defatted as thoroughly as possible with alcohol and petroleum ether and from which 20 to 35 per cent of the dry weight had been removed as material soluble in petroleum ether, remained morphologically intact and acidfast. Such bacilli still contained from 1 to 8 per cent of dry weight of lipid firmly bound to protein in a union, chemical or physical,

of such sort that extraction with fat solvents was impossible. The great variance in the amount present, calculated in percentage of the total dry weight suggested that the union was not a chemical one. This lipid may be rendered extractáble in petroleum ether by treatment during 48 hours of the once-defatted bacilli with normal hydrochloric acid. Simultaneously the integrity of the bacterial cell and its acidfastness disappear. The lipid removable after the acid treatment appeared to be the same in all microörganisms examined. It had the same characteristics as the wax. Similar behavior has been noted regarding fat in starch.

Anderson and Roberts (1930b) give the analyses in Table 39 of the lipid extractable by alcohol, ether, or chloroform from bacilli from three types of animal. They state that considerable fatty material remained unextractable by any means short of destruction of the bacilli.

Table 39. Lipids Separated from Bovine, Avian, and Human Tubercle Bacilli (Anderson and Roberts, 1930b). (Per Cent of Dry Bacteria)

Type of organism	Bovine Strain 523	Avian Strain 531	Human Strain H-37
Approximate no of cultures	1700	2000	2000
Phosphatide, crude	1.53	2.26	6.54
Acetone-soluble fat	3.34	2.19	6.20
Chloroform-soluble wax	8.52	10.79	11.03
Total lipids	13.40	15.26	23.78

It was noted that the Bovine Phosphatide I was easily soluble in ether when first removed, but that exposure to air or drying rendered it insoluble.

The bovine phosphatide fractions after acid hydrolysis in aqueous solution yielded reducing sugars. Bovine Phosphatide II, which was extremely difficult to hydrolyze, showed the same physiological effects as the phosphatide from the human strain—massive formation of tubercular tissue on injection—and also gave positive precipitin tests with tuberculous serum even in high dilution.

Anderson and Roberts (1930a) give the analyses of samples of the phosphatide from avian tubercle bacilli shown in Table 40:

Table 40. Principal Cleavage Products of Avian Phosphatide (Anderson and Roberts, 1930a).

	Analysis 1 (%)	Analysis 2 (%)
Wax-like material	5.93	8.36
Mixture of palmitic and stearic acids	18.42	16.72
Oleic acid after reduction to stearic acid	18.42	17.73
Liquid saturated fatty acid analogous to phthioic acid	14.12	16.72
Glucose	16.50	14.70
Sugar acid obtained as salt with phenylhydrazine	19.96	
Glycerophosphoric acid	6.07	
Magnesium		0.52
Potassium		0.83
Sodium		0 68

The liquid saturated fatty acid was found by titration to have a molecular weight of 303. It was analogous to the peculiar liquid saturated acid described by Anderson (1929a) and Anderson and Chargaff (1929b) and called by them "phthioic acid." It was optically active and possessed the important biological property of stimulating the production of monocytes and epithelioid cells; the subcutaneous injection of the substance into normal healthy animals led to the formation of massive tubercular tissue. Liquid saturated fatty acids with similar chemical, physical, and biological properties have been isolated from every fraction of the lipids of tubercle bacilli, and a sample of the liquid acid purified by fractional distillation of the methyl ester gave two fractions: (1) a liquid without optical or biological activity (except that its sodium salt was bacteriocidal toward *Bacillus leprae*) isomeric with stearic acid which they called tuberculostearic acid; and (2) the true phthioic acid melting at 28°C., isomeric with cerotic acid ($C_{26}H_{52}O_2$), optically active, and characteristically active biologically. They believed that they were dealing with a series of new fatty acids. For the phospholipid from human tubercle bacilli Anderson (1927b) gave the following analysis:

One hundred parts of the phosphatide yielded:

Palmitic acid	30.5 parts
Oleic acid after reduction to stearic acid	12.8 "
Liquid saturated fatty acid	20.9 "
Glucose	13.9 "
Sugar acid	13.8 "
Glycerophosphoric acid	5.4 "

The acetone-soluble material which, in the case of animal tissues, would consist mainly of ordinary fat (triglycerides) and cholesterol was found by Anderson and Chargaff (1929a), in the case of material from human tubercle bacilli, to contain only a small proportion of glycerides. The alcoholic component could not be determined, but was apparently a polyhydric carbohydrate alcohol. The unsaponifiable matter was a liquid which did not contain cholesterol or any substance giving sterol color reactions. The fatty acid mixture consisted of the liquid saturated acids mentioned above to the extent of about 45 per cent of the total. The solid acids consisted mainly of palmitic with a small quantity of stearic and still less cerotic. The unsaturated acid was probably linoleic acid.

In addition to the lipids mentioned above there was found in tubercle bacilli a large amount of a waxy substance. This substance, an alcohol, was studied by Tamura (1913), who found it to be a monohydric alcohol of the formula $C_{29}H_{56}O$ which he called mykol. Mykol was acidfast, and Tamura believed it to be responsible for the acidfast properties of bacteria. Other alcohols have been described by Bürger (1916). Anderson

(1929b) found a large amount of wax-like material in his chloroform extract of the bacilli, representing about 46 per cent of the total lipid and about 11 per cent of the dry weight of the bacteria. It was readily soluble in chloroform or ether but nearly insoluble in alcohol or acetone. Analysis showed that it was not a wax but a complex phosphatide containing a large proportion of carbohydrate. It also contained a fatty acid which caused the formation of tubercular tissue and in addition the commoner fatty acids: palmitic, stearic, and oleic together with cerotic acid. More than half of the substance consisted of an unsaponifiable snow-white powder which possessed both acidic and alcoholic properties and which was strongly acidfast. The water-soluble portion of the hydrolytic products of the wax consisted of glycerophosphoric acid, a mixture of reducing sugars giving pentose reactions, and an unidentified nitrogen compound. Cooper (1930) determined considerable differences between the virulent (S) and avirulent (R) types of tubercle bacilli, the latter containing about twice as much ether-soluble lipid as the former, whereas the S type produced more water-soluble substances.

Anderson and Renfrew (1930) obtained mannose from a sample of tubercle phosphatide. Anderson and Roberts (1930c) found in the human type inosite, mannose, and probably invert sugar; in the avian type there were inosite and mannose. Bloch (1936) found that Anderson's A-3 phosphatide consisted of the magnesium salt of phosphatidic acid along with but not combined with ammonium salts, water-soluble polysaccharides, and waxes.

A yellow pigment, phthiocol ($C_{11}H_8O_3$), m.p. 173-4°C., strongly acid with bright red crystals, was described by Anderson and Newman (1933). Stodola and Anderson (1936) separated phthiocerol from human tubercle bacilli wax, m.p. 73-74°C., $[\alpha]_D = -4.8$, formula $C_{34}H_{67}(OH)_2OCH_3$. It was not found in other bacterial lipids (Reeves and Anderson, 1937).

The haptene fraction of the tubercle bacillus which is active in fixing complement contains salts (magnesium, calcium, sodium) of phosphatidic acids and, when treated with 1*N* sulfuric acid, yields its fatty acids in three to four days. The phosphoric acid remains attached to the polyalcohols. The acids are saturated and make up about 60 per cent of the total fraction. Their average molecular weight is 335 and there is at least one C_{22} acid. Inositol and glycerol are present. Complement fixation is demonstrable with 1/1600 mg. of the haptene (Macheboeuf, Lévy and Faure, 1937).

Bacillus leprae. Uyei and Anderson (1932) isolated the following products, extractable by solvents at room temperature, from *B. leprae*: phosphatide 2.25, acetone-soluble 6.47, chloroform-soluble wax 9.98, polysaccharide 0.92, and dry bacillary residue 80.38. The crude poly-

saccharides contained two different sugars (Newman and Anderson, 1933b). Trehalose was separated and also a non-crystallizing polysaccharide which gave a pentose color reaction and a positive precipitin reaction with immune serum. Two new alcohols, α- and β-leprosol, having phenolic properties, were isolated from the unsaponifiable fraction (Crowder, Stodola and Anderson, 1936). The leprosin from leprosy bacilli was found to be a complex mixture of solid glycerides and waxes. The fatty acids found were myristic, palmitic, stearic, tetracosanic, and a new hydroxy acid, leprosinic acid. The ether-soluble unsaponifiable was *d*-eicosanol-2 and α-octadecanol-2. The acetone-soluble lipid had a deep red-brown color, and was not a glyceride but a trehalose compound. No phthiocol, tuberculostearic or phthioic acid was found, but a new dextrorotatory, branched-chain saturated fatty acid forming lead salts easily soluble in ether was present (Anderson, Reeves and Crowder, 1937).

Typhoid bacillus. Akasi (1939) examined the fatty substance from dried typhoid bacilli from mice and found it to be almost entirely free fatty acid with a small amount of unsaponifiable matter. The fatty acids consisted of 51 per cent solid and 46.4 per cent liquid. The solid acids consisted mainly of palmitic, with some myristic and lauric but no stearic; the liquid acids were mainly oleic with small amounts of palmitoleic, but no linoleic or linolenic.

Enteric bacilli. Eckstein and Soule (1931) examined the products of the growth of *B. coli* on various media. They found that the fatty acids formed were mostly saturated, with an iodine number of 26 to 31, that no cholesterol was formed, and that phospholipid was formed when alanine was the source of nitrogen but not when cystine was the source.

Williams, Bloor and Sandholzer (1939) obtained the following values for various strains of enteric bacteria: total lipid 4.3-7.9 per cent, total fatty acids 2.5-4.6 per cent of dry weight, and iodine number of mixed fatty acids 42-82. Phospholipid constituted the major portion (above 60 per cent) of the total lipid with a fatty acid content of 63-73 per cent. The phosphorus and nitrogen content of the phospholipid was normal with the exception of one strain, which, because of a very low nitrogen content, may have contained phosphatidic acid instead of ordinary phospholipid; or the phospholipids may have been of the type found by Pangborn and Anderson in timothy bacillus (p. 268). No sterols were found.

Yeast

Considerable attention has been given to the content and nature of the lipid of yeast, of which several varieties have been found to form fat

readily. The most complete study of the formation of fat by yeast is that of Smedley-MacLean and her associates. She (1922) found that lipid is present in the yeast cell in two forms: (1) free and extractable; and (2) a combined form difficult to extract, associated with a sterol, and set free by hydrolysis with dilute acids. A free supply of oxygen and a non-nitrogenous medium rich in carbohydrates were conditions producing an increasing percentage of fat in yeast, but this fat was always "bound." It was probably derived from carbohydrate. Smedley-MacLean and Hoffert (1923) found that when yeast was incubated in oxygenated water, part of the carbohydrate originally present disappeared and an increase of fat took place. In the presence of fructose, glucose, and sucrose, fat and carbohydrate were stored up in the yeast cell. The addition of phosphates to the oxygenated sugar solutions produced an increase in the amount of fat stored and a diminution of the amount of carbohydrate. If the solutions were not oxygenated, the increase of fat was small or absent. The hypothesis was advanced that carbohydrate storage in yeast takes place in two ways: (1) as glycogen or some similar compound, giving a reducing sugar on hydrolysis; and (2) as a hexosephosphate, which forms the first stage in the transformation of carbohydrate to fat. Of a number of substances tried (Smedley-MacLean and Hoffert, 1926), ethyl alcohol and sodium acetate were best utilized by the yeast to form carbohydrate and fat; acetaldehyde was not utilized. Bokorny (1915) reported that the fat of yeast could be increased either by using a concentrated medium or by adding toxic agents, hence by lowering the vitality or metabolic rate of the organism. Old yeast cultures were generally much richer in fat than young, active cultures.

Halden (1934) found that as in many higher plants, the carbohydrate $\rightleftharpoons$ fat equilibrium, in yeast is controlled by variations in the water content. When cultivated under conditions which keep the water below 85 per cent, yeast shows an enormous increase in fat and sterol content, twenty times for total lipid and sixty times the normal values for sterols. On solid media, for example nutrient agar, the sterols increase more rapidly than the glycerides. In Frohberg bottom yeast, the ergosterol fraction comprises 80 per cent of the total sterol. The low water content required for fat and sterol enrichment is obtained by pressing the yeast, spreading it on porous plates or intermittently evacuating and admitting alcohol-laden air. In any case, an abundance of air must be supplied. McAnally and Smedley-MacLean (1934) found that Frohberg yeast behaved similarly to brewer's yeast when incubated in solutions of glucose, galactose, fructose, and sucrose; but with maltose, brewer's yeast stores abnormally high carbohydrate. Frohberg yeast forms fat more

readily in solutions of glucose than in those of galactose, fructose, sucrose, or maltose.

Daubney and Smedley-MacLean (1927) found the phospholipid fatty acids more saturated than those of the fat, which is the reverse of what is found in animals (mammals). The fatty acids were mainly palmitic and oleic. Rewald (1930) found in two yeasts which he examined, phospholipid values of 1.25 and 1.37 per cent with fatty acid content of 49.1 and 58 per cent; the water content was 74.5 and 69.4 per cent, respectively. Newman and Anderson (1933a) examined the lipids of yeast, finding that the phospholipids consisted of about 4 parts lecithin to 1 part cephalin, with a fatty acid content of 66 per cent made up of 14 per cent saturated and 86 per cent unsaturated acids. The saturated acids consisted of about equal parts of palmitic and stearic acids. The unsaturated acids belonged to the C_{16} and C_{18} groups; no acids higher than C_{18} were found and only traces of lauric acid. The fat (acetone-soluble) contained 80 per cent unsaturated acids of the C_{16} (25 per cent) and C_{18} (75 per cent) groups, and 20 per cent saturated acids, of which 75 per cent was palmitic and 25 per cent stearic. The unsaponifiable substance consisted of sterols and hydrocarbons. There were none of the peculiar acids such as were found in the tubercle and related bacteria. In fact, the whole make-up of the lipid fraction in yeast was the same as that usually found in plants and had a relatively simple fatty acid composition.

Salisbury and Anderson (1936) separated and purified the lecithin and cephalin and analyzed the pure products. Both yielded 64 per cent fatty acids which were quite similar in both, consisting of 84-86 per cent of liquid acids, which on reduction gave a mixture of palmitic and stearic acids. The solid acids consisted also of these two acids. In lecithin, reduction of the unsaturated acids yielded 63 per cent palmitic and 37 stearic, in cephalin 58 per cent as stearic and 42 as palmitic. The solid acids of cephalin contained 56 stearic and 44 per cent palmitic. The fatty acid composition was thus relatively simple and included none but the most common fatty acids. The aqueous portion of the hydrolysis mixture yielded, in the case of cephalin, aminoethyl alcohol, inactive glycerophosphoric acid, and phosphoric acid; from lecithin were obtained choline, optically active glycerophosphoric acid, and phosphoric acid.

Molds

The formation of lipids by molds and fungi has been studied by a number of investigators. The influence of temperature (Pearson and Raper, 1927), concentration of carbohydrate (Bohn, 1931), and of mineral composition of the medium (Pontillon, 1932; 1933) have been examined.

C. F. Schmidt (1935) has, however, pointed out that the methods used do not allow the separation of processes of growth and processes of fatty acid formation, and it is conceivable that any factor may have a profoundly different effect on the two processes. He was able to separate the two processes by growing the mold to the point of formation of a fully developed mycelium on a suitable medium, then replacing the medium by a second solution containing the substance to be studied. In the absence of nitrogen in the second medium, no more protoplasm is formed, but the mold continues to metabolize actively. In this way, conditions paralleling those in the adult animal body, in which the protoplasm is relatively constant, are obtained and metabolic activities can be studied. He was able to show that the fully developed mycelium of a strain of *Aspergillus niger* placed on fresh glucose solution synthesized fatty acids under conditions in which further growth did not take place. The fatty acid content doubled in three days, the increase being independent of pH between 2.4 and 8.2, as was also the degree of unsaturation of the fatty acids. Under anerobic conditions, there was less fatty acid formation. Iodoacetic acid (0.001*M* concentration) had no effect on the synthesis of fatty acids.

Aspergillus niger, acting on calcium butyrate, produced β-hydroxybutyric, acetoacetic acids, and acetone, but no crotonic acid. The mold cannot grow on calcium crotonate. Calcium valerate yielded β-hydroxyvaleric and methylethyl ketone. Isovaleric yields acetone (Coppock, Subramaniam and Walker, 1928).

With fat as the sole organic nutrient, growth of *Sterigmatocystis nigra* was much more rapid, according as the fatty acids were more unsaturated. Growth was very slow on the fatty acids of butter (iodine number 33) and very rapid on linseed oil (iodine number 180) (Terroine and co-workers, 1927).

Penicillium was found to decompose triolein, linseed, and walnut oils (Oeffner, 1931). Unsaturated compounds, hydroxy acids, and their lactones are formed during the process.

Pontillon (1932, 1933) found that the formation of glycerides was affected qualitatively by the reaction of the medium and quantitatively by its composition. The mycelium utilized its glycerides when nutrients were deficient, and sterols were thus produced. Sterols were used to some extent during sporulation and autolysis. Under the most favorable conditions, the iodine number was low, the mean molecular weight of the fatty acids was high, and the amount of phospholipids high.

Terroine and Bonnet (1927), in a study of the energy relations of the transformation of carbohydrate to fat in *Sterigmatocystis nigra*, a mold, found that the richer the medium in carbohydrate, the greater the

percentage of fat formed. Loss of energy in the transformation from carbohydrate to fat was only about 10 per cent, which agreed well with the values obtained by calculation. In the reverse process, formation of carbohydrate from fat, Terroine, Bonnet and Duquenois (1927) reported a loss of 23 per cent in germination of seeds, and in *Sterigmatocystis nigra*, a loss of 20 to 25 per cent, the loss being lower in the case of the unsaturated than with the saturated acids. Formation of saturated fatty acids from glucose by *Sterigmatocystis nigra* and *Bacillus de la Fleole* resulted in a greater loss of energy than did the formation of unsaturated acids (Terroine and co-workers, 1927). The formation of saturated acids is both the sign and the consequence of high vital activity.

Barber (1929) grew a mold (*Penicillium*) on solutions containing dextrose, sucrose, or pentose (xylose, arabinose) and found that the fat formed was the same in every case: a mixture of glycerides of palmitic, stearic, oleic, and α- and β-linoleic acids. On glycerol, the growth was weaker, but the end product was the same.

Bohn (1931) grew *Sterigmatocystis nigra* on media varying only in the quantity of levulose, and found that the fat formed increased with the concentration of sugar. The loss of energy in the transformation of levulose into fat was extremely small, as was found for glucose also.

In *Blastomyces dermatiditis*, Peck and Hauser (1938) found lipids up to 8-10 per cent of the dry weight, consisting of one-third phospholipid and two-thirds acetone-soluble lipid. The phospholipids contained choline and colamine, and palmitic, stearic, oleic, and linoleic acids with a small amount of carbohydrate. The acetone-soluble portion contained neutral fat, with the same fatty acids as the phospholipid but with less stearic. Ergosterol was the only sterol found in notable amounts. In *Monilia albicans* these authors found total lipid 5.3 per cent, containing phospholipid 3 parts and acetone-soluble 97 parts. Except for a trace of carbohydrate the phospholipid was the ordinary kind. The sterol was ergosterol and the fat contained palmitic, stearic, oleic, and linoleic acids. The lipids were not markedly different from those of higher forms (Peck and Hauser, 1939).

A survey of 61 molds of the genera *Aspergillus* and *Penicillium* (Ward and associates, 1935) showed nine species of the latter and one of the former whose mycelia contained more than 15 per cent of ether-soluble material. *Penicillium javanicum* (Van Beijma) contained up to 41.5 per cent. The amount of fat depended largely on culture conditions, media of 40 per cent glucose content giving mycelia of highest fat content. In addition to fat, *P. javanicum* yielded a complex carbohydrate and a chitinous substance.

The lipid constituents of mold (*Aspergillus niger*) fat were found by

Bernhauer and Posselt (1937) to contain glycerol 6.2 per cent and total fatty acids 67.5 per cent. The fatty acids consisted of 13 per cent saturated and 45.4 per cent unsaturated. The former consisted of palmitic 7.1 per cent, stearic 0.9 per cent, and lignoceric 1.8 per cent. The unsaturated acids were oleic 21.5 per cent, linoleic 23.9 per cent. Unsaponifiable was 12 per cent, of which the only sterol was ergosterol, 1.4 per cent. The iodine number of the fat was 95.1, the solidifying temperature −11 to −12°C. There was thus the usual high content of unsaturated acids. Lignoceric acid was present, but it was not found in yeast fat.

Grafe and Magistris (1925) found that the phosphatides which dialyze out of mold (*Aspergillus oryzae*) contain oleic and palmitic acids and also betaine, choline, adenine, and glucose. Two phosphatides were identified, one unsaturated and containing glucose and betaine, the other containing only palmitic acid and both betaine and choline.

Bibliography

Addis, T., *see* MacKay, E M.
Akasi, S , *J Biochem* (Japan), **29** 13 (1939) p 273
Andersen, D. H. and Sperry, W. M , *J Physiol.*, **90**· 296 (1937). p 221
Anderson, R J., *J Biol. Chem* , **55**· 611 (1923) p 259, 260
J. Am. Chem Soc., **46**: 1450 (1924). p 260
J Biol Chem , **71**· 407 (1927a) p. 262
ibid , **74**: 537 (1927b); **83**. 169 (1929a), **83**. 505 (1929b). p 271
Chem Rev , **29**: 225 (1941) p 264
See also Crowder, J A., Newman, M S., Reeves, R E., Salisbury, L F , Sifferd, R H., Stodola, F H., Uyei, N
Anderson, R J and Chargaff, E , *J. Biol Chem.*, **84**· 703 (1929a), **85**. 77 (1929b). p. 271
Anderson, R. J. and Moore, M G., *J Am. Chem Soc* , **45**: 1944 (1923). p 261
Anderson, R. J and Newman, M S., *J Biol Chem* , **101**. 773 (1933) p 272
Anderson, R. J. and Renfrew, A G., *J. Am Chem. Soc* , **52** 1252 (1930) p 272
Anderson, R J and Roberts, E G., *J. Biol Chem* , **85**. 519 (1930a) p 270
ibid., **85**: 529 (1930b). p. 270
ibid., **89**: 611 (1930c). p. 272
Anderson, R J , Nabenhauer, F P and Shriner, R L , *J Biol Chem* , **71**· 389 (1927). p 261
Anderson, R J , Reeves, R E. and Crowder, J A., *J. Biol Chem* , **121**: 669 (1937). p. 273
Anderson, W E , *see* Mendel, L B , Reed, L L
Anderson, W. E and Mendel, L B., *J. Biol Chem* , **76** 729 (1928) p 236
André, E , *Bull. soc chim biol.*, **7**· 974 (1925) p 242
Anselmino, K J , Hoffman, F and Rhoden, E , *Arch ges Physiol* (*Pflügers*), **237**· 515 (1936). p. 214
Arning, E. and Lippmann, A., *Z. klin. Med.*, **89**· 107 (1920) p 233
Artom, C., *Arch. sci biol.*, **5**: 22 (1923a). p 214
Bull. soc. chim biol , **5**: 872 (1923b) p. 215
Artom, C and Marziani, R , *Bull soc chim biol* , **6**. 713 (1924) p. 214
Atkinson, H V and Lusk, G , *Proc. Nat. Acad Sci* , **5**. 246 (1919). p 236
Ault, W C , *see* Brown, J B
Ault, W C. and Brown, J. B , *J. Biol Chem.*, **107**: 607 (1934). p. 220
Aylward, F. X , *see* Holt, L. E , Jr.
Aylward, F. X. and Holt, L. E., Jr , *J. Biol Chem* , **121**: 61 (1937). p 217
Backlin, E., "Beiträge zur quantitativen Kenntnis der Gehirnlipoide," Inaug -Diss , Upsala, Almqvist 1930. p 207
Bär, R and Jaffé, R., *Z. Konstitutionslehre*, **10**: 321 (1925). p. 220
Baker, L. C., *see* Drummond, J C.
Banks, A. and Hilditch, T. P., *Biochem J* , **25**: 1168 (1931). p. 212
Barber, H. H., *Biochem. J* , **23**: 1158 (1929). p 277
Bardisian, A., *Pediatria*, **29**: 156 (1921). p. 197
Barnes, R H., *see* MacKay, E. M.
Baumann, E. J. and Holly, O. M., *J. Biol. Chem.*, **55**: 457 (1923). p. 220
Bayle, L., *see* Hée, A.
Beeston, A. W. and Channon, H. J., *Biochem. J.*, **30**: 280 (1936). p. 216
Beeston, A. W., Channon, H. J., Loach, J. V. and Wilkinson, H , *Biochem J* , **30**: 1040 (1936). p. 216
Behmel, G., *Z. physiol Chem* , **208**: 62 (1932). p. 234
Belin, P., *Bull. soc chim. biol.*, **8**: 1120 (1926). p. 237
See also Terroine, E.-F.
Benotti, J., *see* Thannhauser, S. J.
Bensley, R. R., *Anat. Record.*, **69**: 341 (1937). p. 245
Bernhauer, K. and Posselt, G., *Biochem. Z.*, **294**: 215 (1937). p. 278

Best, C H, *see* MacLean, D L.
Best, C. H. and Huntsman, M E, *J Physiol*, 75: 405 (1932). p. 216, 218
ibid, 83: 255 (1935). p 215
Best, C. H. and Ridout, J. H, *J Physiol.*, 84: 7P (1935) p. 216
Am. J. Physiol., 122: 67 (1938). p 217
Best, C. H., Channon, H. J and Ridout, J H, *J Physiol.*, 81: 409 (1934) p. 215
Best, C. H., Hershey, J. M. and Huntsman, M E, *J. Physiol*, 75: 56 (1932). p 216
Beumer, H., *Monatsschr. Kinderheilk*, 19: 409 (1921). p. 230
Biochem. Z., 259: 469 (1933). p 262
Bhattacharya, R. and Hilditch, T. P., *Biochem. J.*, 25: 1954 (1931). p. 238
Bickenbach, W and Rupp, H., *Klin Wochschr.*, 10: 63 (1931). p. 224
Bielschowsky, F., *see* Frankel, E
Bierich, R and Lang, A., *Z. physiol. Chem*, 216. 217 (1933). p. 251
Bierich, R, Detzel, A. and Lang, A., *Z. physiol. Chem*, 201: 157 (1931). p. 235
Blanchard, E W, *see* Yeakel, E H
Blatherwick, N. R and associates (Medlar, E. M, Bradshaw, P. J., Post, A. L. and Sawyer, S. D.), *J. Biol. Chem.*, 103: 93 (1933) p 217
Bleibtreu, M, *Arch. ges Physiol (Pflügers)*, 85: 345 (1901). p. 237
Bleyer, B, *see* Diemair, W.
Blix, G., *Z physiol. Chem.*, 219. 82 (1933) p 205
See also Ohlsson, B.
Bloch, K., *Z. physiol. Chem*, 244. 1 (1936). p 272
Bloor, W. R, *J. Biol. Chem.*, 68· 33 (1926) p 252
ibid, 72: 327 (1927). p 201, 250, 252
ibid., 80· 443 (1928). p 198, 204, 209, 210, 219, 248, 252
ibid., 114: 639 (1936). p 199, 200, 201, 250
ibid., 119: 451 (1937). p 256
See also Snider, R H, Williams, C H, Yasuda, M.
Bloor, W. R. and Snider, R H., *J Biol Chem*, 107 459 (1934). p. 250
Bloor, W. R., Okey, R and Corner, G. W, *J. Biol. Chem.*, 86: 291 (1930). p. 197, 224, 251
Bodansky, M, *J. Biol. Chem.*, 109: 615 (1935). p. 203
Bohn, P. R, *Compt. rend. acad sci*, 193: 441 (1931). p 275, 277
Bokorny, T., *Arch. Physiol*, 1915: 305. p. 274
Bolle, A., *Biochem. Z.*, 24: 179 (1910). p 222
Bollman, J. L., *see* Flock, E
Bonnet, R., *see* Terroine, E.-F
Borberg, N. C., *Skand. Arch Physiol.*, 32: 287 (1915). p. 219
Bouisset, L and Soula, C, *Compt rend soc. biol.*, 110· 673 (1932). p 221
Boyd, E. M, *J. Biol. Chem*, 108: 607 (1935); 112: 591 (1936). p. 222
J. Physiol., 91: 394 (1938). p. 223
ibid., 121: 783 (1937). p 257
Boyd, E. M. and Elden, C A, *Endocrinology*, 19· 599 (1935). p. 223
Boyd, E. M. and Wilson, K M, *J. Clin Investigation*, 14: 7 (1935). p. 224
Bradshaw, P. J., *see* Blatherwick, N R
Brown, J. B., *J. Biol. Chem.*, 80. 455 (1928). p. 211
ibid., 97: 183 (1932). p 205
See also Ault, W. C
Brown, J. B and associates (Knouff, R. A., Conlin, M M. and Schneider, B. M.), *Proc. Soc. Exptl. Biol. Med.*, 37: 203 (1937) p 220
Brown, J B. and Ault, W. C., *J Biol Chem.*, 89· 167 (1930). p. 205
Buchanan, R. E. and Fulmer, E. I., "Physiology and Biochemistry of Bacteria," Vol. 1, p. 105, Baltimore, Williams and Wilkins, 1928. p 263
Buchwald, K. W. and Cori, C F., *Proc Soc Exptl Biol. Med.*, 28: 737 (1931). p. 257
Bürger, M, *Biochem Z*, 78: 155 (1916). p. 271
Bürger, M and Oeter, H. D., *Z. physiol Chem.*, 182: 141 (1929a); 184: 257 (1929b). p. 227
Bullard, H H., *Am J. Anat.*, 19: 1 (1916). p. 245
Bullock, W. E. and Cramer, W, *Proc Roy Soc London*, B87: 236 (1914). p. 234
Bunge, E., *Arch. Ophthalmol. (Graefe's)*, 139: 50 (1938). p. 228
Cahane, M., *see* Parhon, C. I.
Cahn, T., *Ann. physiol. physicochim. biol*, 3 4 (1927) p. 202
Caminade, R, Mayer, A. and Vallée, H., *Ann physiol phys-chim. biol*, 3: 89 (1927). p. 257
Campbell, L. K, *see* Long, E R.
Cartland, G. F and Hart, M C, *J. Biol. Chem*, 66· 619 (1925) p. 223
Cathcart, E. P. and Cuthbertson, D. P., *J Physiol*, 72: 349 (1931). p. 228
Cattaneo, L, *Arch. ital. biol.*, 87: 199 (1932) p 225
Chaikoff, I. L., *see* Changus, G. W., Jones, H. B, Kaplan, A., Lorenz, F. W., Perlman, I.
Chaikoff, I. L. and Kaplan, A., *J. Biol. Chem*, 119. 423 (1937). p. 218
Chaikoff, I. L. and Robinson, A., *J. Biol Chem*, 100: 13 (1933). p. 224
Chaikoff, I. L., Gibbs, G. E., Holtom, G. F and Reichert, F. L., *Am. J. Physiol.*, 116: 543 (1936). p. 215
Chamberlain, E. N., *see* Coope, R.
Changus, G. W., Chaikoff, I. L. and Ruben, S., *J. Biol. Chem.*, 126: 493 (1938). p. 206
Channon, H. J., *see* Beeston, A. W., Best, C. H., Chibnall, A. C.
Channon, H. J. and associates, *Biochem. J.*, 31: 1736 (1937a); 31: 2181 (1937b). p. 217
Channon, H. J. and Foster, C. A M., *Biochem. J*, 28: 853 (1934). p. 260
Channon, H. J. and Wilkinson, H., *Biochem. J*, 30 1033 (1936). p. 216
Channon, H. J, Irving, E and Smith, J. A B., *Biochem J*, 28: 1807 (1934). p. 211
Chanutin, A., *see* Ludewig, S
Chanutin, A. and Ludewig, S, *J Biol Chem*, 102: 57 (1933). p 216
J. Lab. Clin Med, 22. 903 (1937) p 232
Chargaff, E., *Z. physiol. Chem.*, 201: 198 (1931); 218. 223 (1933). p. 266
See also Anderson, R. J.
Chauffard, A. and his school (Laroche, G. and Grigaut, A.), *Ann. méd.*, 8: 149 (1920). · p. 220
Chauffard, A., Laroche, G. and Grigaut, A., *Compt. rend. soc. biol.*, 72: 223, 265 (1912). p. 223
Cheng, L. T., *Z. physiol. Chem.*, 201: 209 (1931). p. 222

Chibnall, A. C., *see* Jordan, R. C., Piper, S. H., Smith, J. A. B.
Chibnall, A. C. and associates, *Biochem. J.*, **25**: 2095 (1931a); **25**: 2111 (1931b); **27**: 1885 (1933a); **27**: 1889 (1933b). p. 262
Chibnall, A. C. and Channon, H. J., *Biochem. J.*, **23**: 176 (1929) and previous articles. p. 259
Collin, G. and associates (Drummond, J. C, Hilditch, T. P. and Gunther, E. R), *J. Exptl. Biol.*, **11**: 198 (1934). p. 258
Collison, D L. and Smedley-MacLean, I., *Biochem. J.*, **25**: 606 (1931) p. 262
Conlin, M. M., *see* Brown, J. B.
Coope, R and Chamberlain, E. N., *J. Physiol.*, **60**: 69 (1925). p. 214
Coope, R. and Mottram, V. H., *J. Physiol.*, **49**: 23 (1914). p. 214
Cooper, F B, *J Biol. Chem.*, **88**: 485, 493 (1930). p 272
Coppock, P D, Subramaniam, V and Walker, T. K., *J. Chem Soc*, **1928**: 1422. p. 276
Cori, C. F, *see* Buchwald, K W.
Corner, G W, *see* Bloor, W. R.
Cowdry, E V., *Contributions to Embryology* (Carnegie Inst Washington) VIII: 41 (1918) p. 244, 247
Cowie, D. M. and Magee, M C., *Arch. Internal Med.*, **53**: 391 (1934). p. 230, 231, 232
Cramer, E., *Arch. Hyg*, **16**: 151 (1893). p. 265
Cramer, W., *see* Bullock, W. E.
Crémieu, A, *see* Javillier, M.
Cronheim, C, *see* Loewy, A.
Crowder, J. A., *see* Anderson, R J.
Crowder, J A. and Anderson, R J, *J Biol Chem*, **104**: 399 (1934). p. 266
Crowder, J. A, Stodola, F H. and Anderson, R J., *J Biol Chem*, **114**: 431 (1936). p 273
Cuthbertson, D. P, *see* Cathcart, E. P.
Cutler, C, *see* Deuel, H J
Daubney, C. G. and Smedley-MacLean, I, *Biochem J*, **21**: 373 (1927). p 275
Dean, H. K. and Hilditch, T. P., *Biochem J.*, **27**: 1950 (1933). p. 241, 243
Dechanet, J., *see* Leulier, A
Detzel, A, *see* Bierich, R.
Deuel, H J, Jr, *see* Halliday, N.
Deuel, H. J., Grunewald, C. F and Cutler, C, *Proc Soc Exptl Biol. Med.*, **31**: 309 (1933). p 217
Diemair, W, Bleyer, B and Schmidt W., *Biochem. Z*, **294**: 353 (1937). p 260
Dimter, A., *Z physiol Chem.*, **208**: 55 (1932). p. 234
Ditt, F, *see* Klenk, E
Dorée, C, *Biochem. J*, **4**: 72 (1909). p 197
Dragstedt, L. R, Prohaska, J. v. and Harms, H. P., *Am. J. Physiol.*, **117**: 175 (1936). p. 217
Drummond, J C, *see* Collin, G
Drummond, J. C and Baker, L. C., *J Soc. Chem Ind.*, **48**: 232 (1929). p. 227
Duff, G. L., *Arch Path*, **22**: 161 (1936). p 233
Duquenois, P., *see* Terroine, E.-F.
Eberhard, T. P, *Arch Path.*, **21**: 616 (1936). p. 233
Eckardt, B., *see* Tropp, C
Eckstein, H C., *Proc Soc. Exptl. Biol. Med*, **23**: 581 (1926). p 226
J Biol. Chem, **73**: 363 (1927) p 226
ibid., **81**: 613 (1929) p. 237, 240, 252
See also Tucker, H. F.
Eckstein, H C. and Soule, M. H., *J. Biol. Chem.*, **91**: 395 (1931). p 273
Eckstein, H C. and Wile, U J., *J. Biol Chem.*, **69**: 181 (1926) p 225
Elden, C A., *see* Boyd, E M
Ellis, N R, *see* Spadola, J M.
Ellis, N R and coworkers, *J. Biol. Chem*, **66**: 101 (1925); **69**: 219, 239 (1926a), *U S. Dept. Agr.*, Dept. Bull, **1407**: 63 (1926b). p. 238, 239
Ellis, N. R and Hankins, O. G., *J. Biol. Chem*, **66**: 101 (1925) p 240
Ellis, N. R. and Isbell, H. S., *J Biol. Chem.*, **69**: 219, 239 (1926) p. 252
Ellis, N. R and Zeller, J H., *J Biol. Chem.*, **89**: 185 (1930) p 237, 238, 240
Embden, G. and Lawaczeck, H., *Z. physiol Chem*, **125**: 199 (1923) p 257
Engman, M. F. and Kooyman, D. J., *Arch. Dermatol Syphilol.*, **29**: 12 (1934) p 226
Entenman, C., *see* Lorenz, F. W.
Eppstein, S H., *see* Morgulis, S.
Epstein, E. and Lorenz, K, *Z physiol. Chem*, **192**: 145 (1930) p 230
Erlandsen, A., *Z physiol. Chem*, **51**: 71 (1907). p 245
Evans, H M and Lepkovsky, S, *J Biol. Chem*, **96**: 143 (1932). p. 258
Falk, F, *Biochem. Z*, **13**: 153 (1908). p 208
Faure, M, *see* Macheboeuf, M A.
Fenger, F., *J. Biol Chem.*, **27**: 303 (1916). p. 223
Fex, J., *Biochem. Z.*, **104**: 82 (1920). p. 230
Flock, E, Bollman, J. L. and Mann, F C., *J. Biol. Chem*, **115**: 179 (1936). p 213
Flock, E., Bollman, J. L., Hester, H. R and Mann, F. C, *J Biol Chem*, **121**: 117 (1937). p 216
Foster, C. A. M., *see* Channon, H. J.
Fränkel, E., *see* Thannhauser, S J
Frankel, E. and Löhr, G., *Z. physiol. Chem.*, **218**: 218 (1933) p 209
Frankel, E., Bielschowsky, F. and Thannhauser, S I, *Z. physiol. Chem*, **218**: 1 (1933). p 209
Freytag, F C. and Smith, H. G, *J. Biol. Chem*, **100**: 309 (1933). p. 210
Frouin, A., *Compt. rend. soc biol.*, **84**: 606 (1921). p 265, 269
Fulmer, E I., *see* Buchanan, R. E
Gaál, A. M, *Z. klin. Med*, **113**: 349 (1930). p 233
Gäumann, E., *Ber. deutsch. botan. Ges.*, **53**: 366 (1935), *C. A.*, **30**: 133 (1936) p 260
Gérard, M. P., Moissonier, Mlle. and Welti, Mlle, *Bull soc. chim. biol.*, **14**: 916 (1932). p. 231
Gibbs, C. E., *Am J. Psychiat.*, **5**: 189 (1925). p. 221
Gibbs, G. E., *see* Chaikoff, I. L.
Gillum, H. L., *see* Okey, R.
Gisvold, O., *see* Hall, J. A.
Glick, D., *see* Sobotka, H.
Glikin, W., *Biochem. Z.*, **4**: 235 (1907). p 222, 230
Goris, A. and Liot, A., *Compt. rend. acad. sci.*, **172**: 1622 (1921). p. 263

Gorodisskay, H., *Biochem. Z.*, 164: 446 (1925). p. 207
Grafe, V. and Magistris, H., *Biochem. Z.*, 162: 366 (1925) p. 278
Graham, S. G., *see* Imrie, C. G.
Grigaut, A., *see* Chauffard, A.
Grunewald, C. F., *see* Deuel, H. J.
Guha, K. D., Hilditch, T. P. and Lovern, J. A., *Biochem. J.*, 24: 266 (1930). p. 211
Gunther, E. R., *see* Collin, G.
Haag, F. E., *Arch. Hyg.*, 100: 271 (1928). p. 265
Halden, W., *Z. physiol. Chem.*, 225: 249 (1934). p. 274
Hall, J. A. and Gisvold, O., *J. Biol. Chem.*, 113: 487 (1936). p. 263
Halliday, N. and associates (Deuel, H. J., Jr., Tragerman, L. J. and Ward, W. E), *J. Biol. Chem.*, 132; 171 (1940). p 232
Hankins, O. G., *see* Ellis, N. R.
Hansen, C., *see* Henriques, V.
Harms, H. P., *see* Dragstedt, L. R.
Hart, M. C., *see* Cartland, G. F.
Hart, M. C. and Heyl, F. W., *J. Biol. Chem.*, 70: 663 (1926). p. 223
Hartley, P., *J. Physiol.*, 36: 17 (1907); 38: 353 (1909). p. 211
Hauser, C. R., *see* Peck, R L.
Haven, F. L., *Proc. Soc. Exptl. Biol. Med.*, 32: 734 (1935). p. 219
Am J. Cancer, 27: 95 (1936). p. 236
J. Biol. Chem, 118: 111 (1937a). p 235
Am. J Cancer, 29: 57 (1937b). p 235
J. National Cancer Institute, 1: 205 (1940). p. 236, 264
Hecht, E, *Z. physiol. Chem*, 231: 29 (1935). p. 264
Hée, A. and Bayle, L, *Bull. soc. chim. biol.*, 14: 758 (1932). p 259
Heitz, J., *see* Labbe, M
Helman, R. M., *see* Palladin, A. V.
Henriques, V. and Hansen, C, *Skand. Arch. Physiol.*, 11: 151 (1901). p. 197, 241
ibid., 14: 390 (1903) p 253
Hentschel, H., *Z. Kinderheilk.*, 52: 623 (1932). p 225
Hermstein, —, *Arch Gynäkol*, 124: 739 (1925). p. 223
Herrick, H. T., *see* Ward, G. E.
Hershey, J. M, *see* Best, C H.
Hester, H. R, *see* Flock, E
Heyl, F W., *see* Hart M C
Hiestand, O, *see* Winterstein, E
Hilditch, T. P., *see* Banks, A., Bhattacharya, R, Collin, G, Dean, H K., Drummond, J. C., Guha, K D.
Hilditch, T P. and Paul, S., *Biochem J*, 32: 1775 (1938). p. 239
Hilditch, T P. and Shorland, F B., *Biochem J*, 31: 1499 (1937) p 212
Hinglais, H, *see* Javillier, M
Hodge, H. C, *J. Biol. Chem*, 101: 55 (1933). p. 227
See also MacLachlan, P. L.
Hoffert, D, *see* Smedley-MacLean, I
Hoffman, F., *see* Anselmino, K J
Holly, O M., *see* Baumann, E J.
Holt, L. E, Jr., Aylward, F. X and Timbus, H. G., *Acta Paediat.*, 22: 425 (1937). p. 231
Holt, L. E, Jr, *see* Aylward, F X.
Holtom, G. F., *see* Chaikoff, I. L.
Hopkins, F. G., *Biochem J.*, 19: 787 (1925). p. 252
Hopkins, S. J., *see* Piper, S. H.
Horner, E., *see* Scheff, G.
Horvath, A. A., *Ind. Eng. Chem. News Ed.*, 13: 89 (1935). p. 259
Hueck, W., *see* Wacker, L.
Huntsman, M. E., *see* Best, C. H.
Hyman, L. W., *see* Wadsworth, A.
Imrie, C. G. and Graham, S. G., *J. Biol. Chem.*, 41: xlviii (1920). p. 213
Ingvaldsen, T., *see* Levene, P. A.
Irving, E., *see* Channon, H. J.
Irving, E. and Smith, J. A. B., *Biochem. J.*, 29: 1358 (1935). p. 211
Isbell, H. S., *see* Ellis, N. R.
Iwanow, S., *Biologia Generalis*, 5: 579 (1929). p. 242
Jackson, F. M., *J. Ministry Agr.*, 39: 261 (1923); *C. A.*, 17: 2920 (1923). p. 238
Jaffé, R., *see* Bär, R., Sorg, K.
Jamieson, G. S., "Vegetable Fats and Oils," New York, Chemical Catalog Co., (Reinhold Publishing Corp.), 1932. p. 257
Javillier, M., Crémieu, A. and Hinglais, H., *Bull. soc. chim. biol.*, 10: 327 (1928). p 199
Jensen, L. B., *J. Bact.*, 33: 98 (1937). p. 264
Joelson, J. J. and Shorr, E., *Arch. Internal Med.*, 34: 841 (1924). p 220
Jones, H. B., Chaikoff, I. L. and Lawrence, J. H., *J. Biol. Chem.*, 128: 631 (1939); 133: 319 (1940). p. 236
Jordan, R. C. and Chibnall, A. C., *Ann. Botany*, 47: 163 (1933). p. 259
Jowett, M., *Biochem. J.*, 25: 1991 (1931). p. 230, 235
Kabat, E. A., *see* Lehrman, L.
Kaplan, A., *see* Chaikoff, I. L.
Kaplan, A. and Chaikoff, I. L., *J. Biol. Chem.*, 108: 201 (1935). p. 218
ibid., 120: 647 (1937). p. 219.
Kaufmann, C., *Z. Geburtsh. Gynekol.*, 91: 668 (1927). p. 224
Kaufmann, C. and Raeth, K., *Arch. Gynäk.*, 130: 128 (1927). p. 223, 251
Kawaguchi, M., *Nagoya J. Med. Sci.*, 10: 348; *C. A.*, 31: 7099 (1937). p. 226
Kawamura, R., "Neue Beiträge zur Morphologie und Physiologie der Cholesterin-steatose," Jena, G Fischer, 1927. p. 246
Kerr, R. H., *J. Am. Chem. Soc.*, 49: 2046 (1927). p. 229
Kimura, K., *see* Tsujimoto, M.
Kleinmann, H., *Arch. path. Anat. (Virchow's)*, 282: 613 (1931). p. 230, 232

Klenk, E., *Z. physiol. Chem.*, **185**: 169 (1929). p. 205
ibid., **217**: 228 (1933a) p. 210
ibid., **221**: 259, 264 (1933b) p. 211
ibid., **235**: 24 (1935) p. 232
Klenk, E and Ditt, F., *Z physiol. Chem.*, **226**: 213 (1934) p. 199
Klenk, E and Schoenebeck, O v, *Z physiol. Chem.*, **194**: 191 (1931). p. 205
ibid., **209**: 112 (1932). p. 211, 212
Knock, G., *see* Okey, R.
Knouff, R. A., *see* Brown, J. B.
Knudson, A., *see* Randles, F. S.
Koch, W. and Koch, M. L., *J. Biol. Chem.*, **15**: 423 (1913). p. 207
Koganei, R., *J. Biochem. (Japan)*, **1**: 353 (1922). p. 268
Komatsu, S., *see* Levene, P. A.
Kooyman, D J., *see* Engman, M. F.
Koppenhoefer, R. N., *J. Biol. Chem.*, **116**: 321 (1936). p. 225
Krasnow, F., *see* Rosen, I.
Krause, A. C., *Am. J. Physiol.*, **110**: 182 (1934). p. 228
Arch. Ophthalmol. N.S., **13**: 1022 (1935). p. 228
Labbe, M., Nepveux, F. and Heitz, J., *Compt rend. soc biol.*, **88**: 1131 (1923). p. 234
Landé, K. E. and Sperry, W. M., *Arch. Path.*, **22**: 301 (1936) p. 234
Lanfranchi, E., *Arch. sci. biol.* (Italy), **24**: 120 (1938). p. 204
Lang, A., *Z. physiol Chem.*, **246**: 219 (1937) p. 207, 214
See also Bierich, R.
Laroche, G., *see* Chauffard, A.
Larson, L. W. and Larson, W. P., *J. Infectious Diseases*, **31**: 407 (1922). p. 265
Laszt, L., *see* Verzár, F.
Laurie, P., *Bull. soc. chim. biol.*, **15**: 418 (1933). p. 208
Lawaczeck, H., *see* Embden, G.
Lawrence, J. H., *see* Jones, H. B.
Le Breton, E., *Bull. soc. chim biol.*, **3**: 539 (1921). p. 199, 247, 249
Lehrman, L., *see J. Am. Chem. Soc.*, **52**: 808 (1930). p. 257
See also Taylor, T. C.
Lehrman, L. and Kabat, E A., *J. Am Chem Soc.*, **59**: 1050 (1937) p. 258
Lemeland, P., *Bull. soc. chim. biol.*, **3**: 134 (1921), **4**: 300 (1922), **5**: 110 (1923) p. 249
Lepkovsky, S., *see* Evans, H. M.
Leulier, A. and Dechanet, J., *Compt. rend soc biol*, **95**: 1369 (1926). p. 220
Levene, P. A. and Ingvaldsen, T, *J. Biol Chem*, **43**: 359 (1920) p. 213
Levene, P. A. and Komatsu, S., *J Biol Chem.*, **39**: 91 (1919) p. 223
Levene, P. A. and Rolf, I. P, *J Biol. Chem*, **54**: 91 (1922). p. 204
ibid., **62**: 759 (1924). p. 258
Levene, P. A. and Simms, H. S, *J. Biol. Chem*, **48**: 185 (1921). p. 213, 252
Lévy, G., *see* Macheboeuf, M. A
Ling, S. M., *see* Wiersuchowski, M.
Liot, A., *see* Goris, A.
Lippmann, A., *see* Arning, E.
Loach, J. V., *see* Beeston, A. W
Lobstein, J. E., *see* Terroine, E.-F.
Lockwood, L. B, *see* Ward, G. E.
Lohr, G., *see* Fränkel, E.
Loewy, A and Cronheim, C, *Biochem Z.*, **257**: 267 (1933) p. 213
Long, E R, *Am. Rev. Tuberc.*, **6**: 642 (1923). p. 269
Long, E. R. and Campbell, L. K., *Am. Rev Tuberc.*, **6**: 636 (1922). p. 265
Lorenz, F. W., Chaikoff, I. L. and Entenman, C., *J. Biol Chem*, **123**: 577 (1938). p. 213
Lorenz, K., *see* Epstein, E.
Lovern, J. A., *Biochem. J.*, **24**: 866 (1930). p. 211
See also Guha, K. D
Ludewig, S., *see* Chanutin, A.
Ludewig, S. and Chanutin, A., *J. Biol Chem.*, **115**: 327 (1936). p. 256
Lusk, G., *see* Atkinson, H. V.
Lyons, R. E., *Arch. Hyg.*, **28**: 30 (1897). p. 265
McAnally, R. A. and Smedley-MacLean, I., *Biochem. J.*, **28**: 495 (1934). p. 274
McConnell, K. P. and Sinclair, R. G., *J. Biol. Chem.*, **118**: 123 (1937a). p. 224
ibid., **118**: 131 (1937b). p. 205, 207, 224
Macheboeuf, M. A., Lévy, G. and Faure, M., *Compt. rend acad sci*, **204**: 1843 (1937) p. 272
MacKay, E. M. and associates (MacKay, L. L. and Addis, T.), *Am. J. Physiol*, **86**: 459 (1928). p. 257
MacKay, E. M. and Barnes, R. H., *Proc Soc. Exptl. Biol. Med.*, **38**: 410 (1938a). p. 217
ibid., **38**: 803 (1938b). p. 214
MacLachlan, P. L, *Proc. Soc Exptl. Biol. Med.*, **34**: 31 (1936a). p. 214
J. Biol. Chem., **114**: 185 (1936b). p. 262
MacLachlan, P. L. and Hodge, H C, *J Biol. Chem*, **127**: 721 (1939). p. 214
MacLean, D. L. and Best, C. H, *Brit. J Exptl. Path*, **15**: 173 (1934). p. 215
McQuarrie, I, *see* Stoesser, A. V.
Magee, M. C., *see* Cowie, D. M.
Magistris, H., *see* Grafe, V.
Magistris, H. and Schäfer, P., *Biochem. Z.*, **214**: 401 (1929). p. 258
Mann, F. C., *see* Flock, E.
Marino, S., *Arch. farmacol. sper.*, **55**: 183 (1933). p. 221
Marziani, R., *see* Artom, C.
May, O. E., *see* Ward, G. E.
May, R. M., *Bull. soc. chim. biol.*, **12**: 934 (1930). p. 208
Mayer, A., *see* Caminade, R.

Mayer, A. and Schaeffer, G., *J physiol. path. gén*, **15**: 510, 534 (1913a). p. 245
ibid., **15**: 773 (1913b); **16**: 1, 23 (1914a) p. 247
ibid., **16**: 325, 344 (1914b). p 248
Compt. rend. acad. sci., **159**: 102 (1914c). p 249
Mayer, A, Rathery, F. and Schaeffer, G., *Compt. rend soc. biol.*, **76**: 398 (1914). p. 244
Medlar, E M., *see* Blatherwick, N. R.
Mendel, L. B., *see* Anderson, W. E., Reed, L L.
Mendel, L. B. and Anderson, W. E, *Science*, **64**: 384 (1926). p. 239
Mentzer, S. H., *Am J. Path.*, **1**: 383 (1925). p. 228
Merz, W., *Z physiol. Chem.*, **196**: 10 (1931). p. 205
Meyerhof, O, *Arch ges Physiol. (Pflügers)*, **199**: 531 (1923). p 252
Mikulicz-Radecki, F. v., *Arch. Gynäkol.*, **116**: 203 (1923) p. 223
Miura, K., *J. Biochem. (Japan)*, **25**: 579 (1937). p. 224
Moissonier, Mlle, *see* Gérard, M. P.
Monasterio, G., *Biochem. Z.*, **218**: 331 (1930). p. 213
Moore, M. G., *see* Anderson, R. J.
Morgulis, S., Wilder, V. M., Spencer, H. C. and Eppstein, S. H, *J. Biol. Chem*, **124**: 755 (1938). p. 203
Mottram, V. H., *J. Physiol.*, **50**: 380 (1916). p. 219
See also Coope, R
Moulton, F. R. and Trowbridge, P F., *J. Ind Eng. Chem.*, **1**: 761 (1909). p. 239
Nabenhauer, F. P., *see* Anderson, R. J
Nelson, J. M, *see* Taylor, T. C
Nepveux, F, *see* Labbe, M
Newman, M. S, *see* Anderson, R J.
Newman, M S and Anderson, R J, *J Biol Chem.*, **102**: 219, 229 (1933a) p. 275
Z. physiol. Chem, **220**: 1 (1933b). p 273
Nichols, R. R., *see* Wadsworth, A.
Nishimoto, U., *see* Suzuki, B.
Oeffner, H, *Botan Arch*, **33**: 172 (1931). p 276
Oeter, H D, *see* Bürger, M
Ohlsson, B. and Blix, G., *Skand Arch. Physiol*, **69**: 192 (1934) p. 213, 218
Okey, R, *Proc Soc Exptl Biol Med*, **30**. 1003 (1923). p 216
See also Bloor, W R.
Okey, R. and Yokela, E, *J Nutrition*, **11**: 463 (1936) p 216
Okey, R, Gillum, H L and Yokela, E, *J Biol Chem*, **107**: 207 (1934) p 217
Okey, R, Yokela, E and Knock, G., *Proc Soc Exptl Biol. Med*, **31**: 507 (1934). p. 216
Page, I H, *see* Pasternak, L
Page, I H. and Rudy, H, *Z physiol Chem*, **205**: 115 (1932). p 205
Palladin, A. V, Rashba, E I and Helman, R M., *Ukrain Biochem Zhur*, **8**: no. 1, 5 (1935a); *C. A.*, **30**: 5277 (1936) p 206
Ukrain Biochem Zhur, **8**: no 1, 27 (1935b); *C A*, **30**: 5277 (1936). p 206
Pangborn, M C. and coworkers, *J Biol Chem*, **94**: 465 (1931); 98. 43 (1932). p. 268
Paras, E. M, *Philippine J. Sci*, **66**: 155 (1938); *C. A.*, **33**: 4311 (1939). p 231
Parhon, C I and Cahane, M., *Compt. rend. soc biol*, **107**: 836 (1931). p. 220
Parhon, C. J. and Parhon, M, *Compt rend soc biol.*, **88** 231 (1923) p. 197
Pasternak, L and Page, I H., *Biochem Z*, **282**: 282 (1935). p 203
Paul, S., *see* Hilditch, T P
Pearson, L. K and Raper, H S, *Biochem J.*, **21**: 875 (1927). p 243, 275
Peck, R L. and Hauser, C R., *J Am Chem Soc.*, **60**: 2599 (1938); **61**: 281 (1939). p. 277
Perlman, I. and Chaikoff, I. L, *J. Biol Chem*, **127**: 211 (1939). p. 209, 218
Petri, K. A, *see* Stoesser, A. V
Pfeiffer, G., *Biochem Z.*, **231**: 239 (1931a). p. 221
ibid., **235**: 97 (1931b) p 206
Pigulevskii, G. V., *J. Russ Phys Chem Soc*, **48**: 324 (1916), *C A*, **11**: 543 (1917) p 242
Piper, S H and associates (Chibnall, A C, Hopkins, S. J., Pollard, A., Smith, J. A B. and Williams, E. F.), *Biochem. J.*, **25**: 2072 (1931). p 262
Pollard, A., *see* Piper, S. H.
Pontillon, C., *Rev. gén. botan.*, **44**: 417, 465, 526 (1932); **45**: 20 (1933). p. 275, 276
Posselt, G., *see* Bernhauer, K
Post, A. L, *see* Blatherwick, N. R.
Present, C. H., *see* Ralli, E P
Prohaska, J. v., *see* Dragstedt, L. R.
Quagliariello, G., *Arch. internat. physiol.*, **16**: 239 (1921). p. 200
Raeth, K., *see* Kaufmann, C.
Ralli, E. P., Rubin, S. H. and Present, C. H., *Am. J. Physiol.*, **122**: 43 (1938). p 217
Randall, L. O., *J. Biol. Chem.*, **124**: 481 (1938a). p. 204
ibid., **125**: 723 (1938b) p. 208
Randles, F. S. and Knudson, A., *J. Biol. Chem.*, **76**: 89 (1928). p. 220
Raper, H. S., *see* Pearson, L. K.
Rashba, E. I., *see* Palladin, A. V.
Rathery, F., *see* Mayer, A.
Reed, L. L., Anderson, W. E and Mendel, L. B, *J. Biol. Chem.*, **96**: 313 (1932). p 240
Reeves, R. E., *see* Anderson, R. J.
Reeves, R. E. and Anderson, R. J., *J. Biol. Chem.*, **119**: 535 (1937). p. 272
Reichert, F. L., *see* Chaikoff, I. L.
Reiner, M., *see* Sobotka, H.
Reinstein, H., *see* Thannhauser, S. J.
Renfrew, A G., *see* Anderson, R. J
Rewald, B, *Biochem Z.*, **216**: 11 (1929). p. 259
ibid., **218**: 481 (1930). p. 275
J. Soc. Chem. Ind., **56**: 403T (1937). p. 259
Rhoden, E., *see* Anselmino, K. J.
Ridout, J. H., *see* Best, C. H.

Roberts, E. G., see Anderson, R. J.
Robinson, A., see Chaikoff, I. L.
Roffo, A. H., Neóplasmes, 7: 344 (1928) p. 225
Bol. inst. méd. exptl. estud. cancer, 8: 32 (1931). p. 226
J. physiol. path. gén., 30: 345 (1932). p. 226
Rolf, I. P., see Levene, P. A.
Rosen, I. and Krasnow, F., Arch. Dermitol. Syphilol., 26: 48 (1932). p. 232
Rosenbloom, J., J. Biol. Chem., 14: 291 (1913). p. 245
Rosenfeld, G., Ergeb. Physiol., 1: 651 (1902). p. 238
Rossi, A., Z. physiol. Chem., 231: 115 (1935). p. 209
Rowland, R. S., Oxford Medicine, 4: 214 (3) (1927–36). p. 231
Ruben, S., see Changus, G. W.
Rubin, S. H., see Ralli, E. P.
Rubow, V., Arch. exptl. Path. Pharmakol., 52: 173 (1905). p. 245
Rudy, H., see Page, I. H.
Rupp, H., see Bickenbach, W.
Salgues, R., Compt. rend. soc. biol., 124: 923 (1937). p. 227
Salisbury, L. F., J. Biol. Chem., 117: 21 (1937). p. 263
Salisbury, L. F. and Anderson, R. J., J. Biol. Chem., 112: 541 (1936). p. 275
ibid., 129: 505 (1939). p. 229
Salit, P. W., Arch. Ophthalmol., N. S., 5: 354 (1931). p. 227
Sandholzer, L. A., see Williams, C. H.
Sando, C. E. and associates, J. Biol. Chem., 56: 457 (1923); 98: 103 (1932) p. 262
Sawyer, S. D., see Blatherwick, N. R.
Schafer, P., see Magistris, H.
Schaeffer, G., see Mayer, A.
Scheff, G. and Horner, E., Biochem. Z., 248: 181 (1932). p. 215
Schmidt, C. F., Jr., J. Biol. Chem., 110: 511 (1935). p. 214, 276
Schmidt, L. H., Am. J. Physiol., 111: 138 (1935). p. 203
Schmidt, W., see Diemair, W.
Schneider, B. M., see Brown, J. B.
Schoenebeck, O. v., see Klenk, E.
Schoenheimer, R., Z. physiol. Chem., 160: 61 (1926); 177: 143 (1928). p. 234
Science, 74: 579 (1931). p. 197
Z. klin. Med., 123: 749 (1933). p. 233
Shioji, E., J. Biochem. (Japan), 4: 43 (1924). p. 254
Shirahama, K., see Takahashi, E.
Shorland, F. B., see Hilditch, T. P.
Shorr, E., see Joelson, J. J.
Shriner, R. L., see Anderson, R. J.
Sieber, N., Biochem. Z., 23: 304 (1910). p. 231
Sifferd, R. H. and Anderson, R. J., Z. physiol. Chem., 239: 270 (1936). p. 264
Simms, H. S., see Levene, P. A.
Sinclair, R. G., J. Biol. Chem., 82: 117 (1929). p. 227, 254
ibid., 86: 579 (1930). p. 213, 253
ibid., 92: 245 (1931). p. 219, 253
ibid., 95: 393 (1932a). p. 202, 250, 252, 254
ibid., 96: 103 (1932b). p. 252
Am. J. Physiol., 103: 73 (1933). p. 224
J. Biol. Chem., 111: 261 (1935a). p. 202, 255
ibid., 111: 275 (1935b). p. 252, 255
ibid., 111: 515 (1935c). p. 250, 255
ibid., 134: 89 (1940). p. 222
See also McConnell, K. P.
Slifer, E. H., Physiol. Zoöl., 5: 448 (1932). p. 229
Slonaker, J. R., Anat. Record, 2: 116 (1908). p. 256
Small, J. C., J. Lab. Clin. Med., 1: 809 (1916). p. 233
Smedley-MacLean, I., Biochem. J., 16: 370 (1922). p. 263, 274
See also Collison, D. L., Daubney, C. G., McAnally, R. A.
Smedley-MacLean, I. and Hoffert, D., Biochem. J., 17: 720 (1923); 20: 343 (1926). p. 274
Smedley-MacLean, I. and Thomas, E. M., Biochem. J., 14: 483 (1920). p. 263
Smith, H. G., see Freytag, F. C.
Smith, J. A. B., see Channon, H. J., Irving, E., Piper, S. H.
Smith, J. A. B. and Chibnall, A. C., Biochem. J., 26: 218 (1932). p. 259
Snider, R. H., J. Biol. Chem., 116: 503 (1936). p. 201
See also Bloor, W. R.
Snider, R. H. and Bloor, W. R., J. Biol. Chem., 99: 555 (1933). p. 201
Sobotka, H., "The Chemistry of the Sterids," Baltimore, Williams and Wilkins Co., 1938. p. 198
Sobotka, H. and associates (Glick, D., Reiner, M. and Tuchman, L.), Biochem. J., 27: 2031 (1933). p. 232
Sorg, K., Z. Konstitutionslehre, 10: 67 (1924). p. 222
Z. physiol. Chem., 182: 97 (1929). p. 257
Sorg, K. and Jaffé, R., Zentr. allgem. Path., 35: 353 (1924). p. 220
Soula, C., see Bouisset, L.
Soule, M. H., see Eckstein, H. C.
Spadola, J. M. and Ellis, N. R., J. Biol. Chem., 113: 205 (1936). p. 238
Spencer, H. C., see Morgulis, S.
Sperry, W. M., J. Biol. Chem., 114: 125 (1936). p. 246
See also Anderson, D. H., Landé, K. E.
Stephenson, M. and Whetham, M. D., Proc. Roy. Soc. London, B93: 262 (1922). p. 267
Stodola, F. H., see Crowder, J. A.
Stodola, F. H. and Anderson, R. J., J. Biol. Chem., 114: 467 (1936). p. 272
Stoesser, A. V., Petri, K. A. and McQuarrie, I., Proc. Soc. Exptl. Biol. Med., 32: 761 (1935). p. 205
Subramaniam, V., see Coppock, P. D.

Susuki, B., *see* Yokoyama, Y.
Susuki, B. and Nishimoto, U., *Proc Imp. Acad (Tokyo)*, **6**: 262 (1930) p. 258
Takahashi, E., Shirahama, K. and Tasi, S., *J. Chem. Soc. Japan*, **54**: 619 (1933); *C. A.*, **27**, 5778 (1933). p. 258
Takizawa, T., *Gaun*, **31**: 370 (1937); *C. A.*, **32**: 3479 (1938). p 235
Tamura, S., *Z. physiol Chem.*, **87**: 85 (1913) p 271
Tanaka, K., *J. Biochem (Japan)*, **17**: 483 (1933). p. 263
Tasi, S., *see* Takahashi, E
Taylor, T. C and Lehrman, L., *J. Am. Chem. Soc*, **48**: 1739 (1926). p. 258
Taylor, T C. and Nelson, J M, *J Am. Chem Soc*, **42**: 1726 (1920) p 257
Terroine, E -F, *J physiol path. gén*, **16**. 408 (1914). p 246
"Contribution à la connaissance de la physiologie des substances grasses et lipoidiques," Paris, Masson et Cie, 1919 p 197, 202
Terroine, E -F. and Belin, P, *Bull. soc chim biol*, **9**: 12 (1927) p 249,252, 253
Terroine, E -F. and Bonnet, R, *Bull. soc chim biol*, **9**: 588 (1927). p 276
Terroine, E -F. and coworkers, *Bull soc chim. biol*, **9**. 605 (1927). p 243, 276, 277
ibid, **12**: 657, 674 (1930). p 228
Terroine, E -F and Lobstein, J. E, *Bull soc chim. biol.*, **5**: 182 (1923). p 263, 269
Terroine, E.-F and Weill, J., *J. physiol path gén*, **15**: 549 (1913) p. 245, 246
Terroine, E.-F, Bonnet, R and Duquenois, P, *Bull. soc chim biol*, **9**: 597 (1927). p 277
Teunissen, P. H, *Z physiol Chem*, **248**: 142 (1937). p 232
Thannhauser, S I, *see* Fränkel, E
Thannhauser, S J. and associates (Benotti, J, Walcott, A and Reinstein, H.), *J. Biol Chem*, **129**: 717 (1939). p 199
Thannhauser, S. J and Fränkel, E, *Z physiol Chem*, **203** 183 (1931). p. 210
Thaysen, T E H, *Biochem Z*, **62** 115 (1914) p 246
Theis, E R, *J Biol. Chem*, **76**: 107 (1928) p 210, 247, 252, 253
ibid, **82**: 327 (1929). p 230
Thomas, E. M, *see* Smedley-MacLean, I
Thudichum, J L W, "Die chemische Konstitution des Gehirns, des Menschen, und der Tiere," Tübingen, Pietzcker, 1901 p 203
Timbus, H G, *see* Holt, L E, Jr
Timon-David, J, *Bull soc chim biol*, **12**: 395 (1930). p 229
Tornsi, D., *Arch sci. biol (Italy)*, **19**: 398 (1934) p 213
Tragerman, L J, *see* Halliday, N
Tropp, C and Eckardt, B, *Z physiol Chem*, **238**: 31 (1936a). p 234
ibid., **243**: 38 (1936b) p 232
Tropp, C and Wiedersheim, V., *Z physiol Chem*, **222** 39 (1933) p 221
Trowbridge P F, *see* Moulton, F R
Tsujimoto, M., *J Soc Chem Ind*, **51**: 317 (1932) p 210
Tsujimoto, M and Kimura, K, *Chem. Umschau Fette, Öle, Wachse, Harze*, **35** 317 (1928); *C A*, **22**: 1245 (1928) p 210
Tuchman, L, *see* Sobotka, H
Tucker, H F and Eckstein, H C, *J. Biol Chem*, **121** 479 (1937) p 216
Turner, K., *Biochem J.*, **25**: 49 (1931). p 219
Tuthill, C. R, *Arch. Path*, **25**. 336 (1938) p 204
Uhnoo, B, *Z physiol Chem*, **256** 104 (1938) p 227
Updegraff, H., *Proc. Soc Exptl Biol Med*, **29** 964 (1932) p 228
Uyei, N and Anderson, R J, *J Biol Chem*, **94** 653 (1932). p 272
Vallée, H, *see* Caminade, R
Verzár, F and Laszt, L, *Biochem Z*, **270** 24 (1934). p 227
Vickery, J R, *J Council Sci Ind Research*, **9** 107 (1936) p 264
Wacker, L and Hueck, W, *Arch exptl Path Pharmakol*, **71**: 373, 416 (1913). p. 197
Wadsworth, A, Hyman, L W and Nichols, R. R, *Am J Path*, **11**. 419 (1935) p 215
Walcott, A, *see* Thannhauser, S J
Walker, T K, *see* Coppock, P D
Ward, G E and associates (Lockwood, L B, May, O. E and Herrick, H T.), *Ind Eng Chem*, **27** 318 (1935) p 277
Ward, W E, *see* Halliday, N.
Watanabe, H., *J Biochem (Japan)*, **2** 369 (1923) p 224
Weill, J, *see* Terroine, E -F
Welch, A D, *Proc. Soc Exptl Biol Med*, **35**. 107 (1936). p 218
Welti, Mlle., *see* Gérard, M P
Wertheimer, E, *Arch. Exptl Path. Pharmakol*, **160**: 177 (1931). p. 215
Wesson, L. G., *J. Biol Chem*, **65**: 235 (1925) p. 211
Whetham, M D., *see* Stephenson, M.
Wiedersheim, V, *see* Tropp, C.
Wierzuchowski, M and Ling, S M, *J Biol Chem*, **64** 697 (1925) p 237
Wilder, V M, *see* Morgulis, S
Wile, U. J., *see* Eckstein, H. C
Wilkinson, H., *see* Beeston, A. W., Channon, H J.
Williams, C H, Bloor, W. R and Sandholzer, L. A., *J Bact.*, **37**: 301 (1939). p. 273
Williams, E. F, *see* Piper, S. H.
Wilson, K. M., *see* Boyd, E. M
Windaus, A, *Z. physiol. Chem*, **65**: 110 (1910). p. 230
Winfield, G., *J. Physiol.*, **49**: 171 (1915). p. 257
Winterstein, E. and Hiestand, O, *Z. physiol. Chem*, **54**: 288 (1908). p. 258
Yasuda, M., *J. Biochem. (Japan)*, **26**: 203 (1937) p. 204
Yasuda, M. and Bloor, W. R, *J. Clin. Investigation*, **11**: 677 (1932). p. 235, 251
Yeakel, E. H. and Blanchard, E. W., *J. Biol. Chem*, **123**: 31 (1938). p. 220
Yokela, E., *see* Okey, R
Yokoyama, Y. and Susuki, B., *Proc. Imp. Acad. (Tokyo)*, **7**: 12 (1931) p. 258
ibid., **8**: 183 (1932). p. 205
Zeller, J. H., *see* Ellis, N. R.

Chapter V

Lipid Metabolism

Introduction

Metabolism is defined as the sum of all changes undergone by substances in the living body; it includes the processes of synthesis of elementary as well as complex tissue constituents, of interchange between various groups of body constituents, of changes undergone in the final stages of combustion for energy production, and of excretion of waste. The chemistry of these changes in the lipids is being slowly traced out; but, although there has been a large volume of experimentation and an even greater amount of theorizing and speculation, it is not yet possible in most cases to be certain of what happens. Nevertheless, the processes involved, forming as they do the basis of life itself, cannot fail to challenge the interest and best efforts of every chemist. Less is known about the metabolism of the lipids than of the other great groups of substances, partly because there has been less effort expended on them and partly because they are inherently difficult to approach. The essential constituents of the lipids, mainly fatty acids and sterols, are concentrated, *i.e.*, high-calorie, low-oxygen compounds insoluble in water; and, since they originate in and are finally degraded to the universal raw materials, carbon dioxide and water, without detectable intermediate stages, the processes of synthesis and of combustion are more difficult to follow than is the case with the amino acids and sugars. The whole of our knowledge at present constitutes a picture puzzle of which only a few parts are available. Nevertheless, it is interesting and instructive to try to fit these parts together to get some idea of what the complete picture might be.

The formation of fatty acids and fat from other food substances in animals has been demonstrated. In the case of carbohydrate, it is easy to demonstrate by feeding experiments and is a universally accepted fact. The formation of fat from protein is probable, since it has been shown in the diabetic animal that protein yields carbohydrate or its equivalent to the extent of about two-thirds of its molecule. The demonstration of the change of protein to carbohydrate is difficult because of the metabolic peculiarities of protein, but it has been accomplished rather satisfactorily and the physiological formula for its transformation has been stated (Atkinson, Rapport and Lusk, 1922). The reverse changes—

the formation of carbohydrate from fat or fatty acid, and of protein from fat—although to be expected as a reversal of these processes, have not been demonstrated. The combustion of the fatty acid, the basic constituent of the lipids, is provisionally accounted for as regards the final steps and possibly the early stages, but the intermediate stages are unknown. The most important animal sterol, cholesterol, can be synthesized and excreted by the animal body. Whether it can be burned in significant amounts seems doubtful. The ordinary phospholipids, lecithin and cephalin, are readily synthesized and in fact appear to be regular stages in the metabolism of the fats. Cholesterol esters are also synthesized in the early stages of fat metabolism.

Physiological Synthesis of Lipids

Synthesis of Neutral Fat

The early history of the research on fat synthesis involves the names of Dumas, who in 1840 believed that the stored fat of animals originated only in the fat of the food; of Liebig, who suggested that it was formed from carbohydrates; and of Voit and von Pettenkofer, who thought they had proved that the fat was formed from protein, until Pflüger in 1892 demonstrated the fallacy of one of their important assumptions. In the meantime, Lawes and Gilbert, from 1860 to 1866, working with farm animals in England, furnished evidence to show beyond reasonable doubt that carbohydrate was an important source of body fat. Their work has been abundantly confirmed since that time, and the formation of fat from carbohydrate is now an accepted fact.

From protein

The formation of fat from protein, which Voit and von Pettenkofer believed that they had shown, proved very difficult to demonstrate, first because it is not easy to get protein sufficiently free of fat to be acceptable for this type of experiment, and secondly because protein stimulates metabolism to such an extent that it brings about its own destruction. Conditions under which the formation of fat from protein occurs have been stated by Atkinson, Rapport and Lusk (1922) as follows:

When the glycogen reservoirs are low, ingestion of large quantities of protein (lean meat) results in the deposition of glycogen; continued ingestion of meat results in the deposition of both glycogen and fat; and the forced feeding of excess meat results in extensive fat formation. After a carbohydrate meal the evening before, ingestion of meat causes the production of fat. From this it would be inferred that fat formation from protein takes place through carbohydrate as an intermediate stage. Anderson and Mendel (1928) and Eckstein (1929) both found that the

fat formed from protein by their experimental animals was the same kind of fat as that formed from carbohydrate. Longenecker (1939) found that the fat stored in rats fed either 95 per cent sucrose or 95 per cent protein was very similar, and markedly different from that deposited when there was 5 per cent fat in the diet. It contained 40-45 per cent C_{16} acids as compared with 25-30 per cent on the 5 per cent fat diet; 50 to 55 per cent C_{18} acids as compared with 67-68 per cent on the 5 per cent fat diet; also 13-16 per cent hexadecenoic (palmitoleic) as compared with 4 per cent on the 5 per cent fat diet. These findings confirm the essential similarity of fats formed from protein and carbohydrate. He found also that utilization of depot fat by fasting did not change the composition of the remainder; therefore there was no selection during use. There was a tendency to restrict the saturated acids to 36 per cent.

From carbohydrate

Theories as to the probable mechanism of the formation of fat from carbohydrate (or from protein, since in its formation from protein it goes through a carbohydrate stage) are based on the observation that, almost without exception, the naturally occurring fatty acids contain an even number of carbons, *i.e.*, are multiples of two, which points to the probability that they originate in some substance containing two carbon atoms. The accepted conception of fatty acid catabolism, the β-oxidation hypothesis, requires the breakdown of the chain by pairs of carbon atoms.

The substance first suggested was acetaldehyde (Leathes, 1906), and this substance continues to hold the field as the theoretical starting point in fatty acid synthesis. Its ready formation from lactic acid and the fact that lactic acid when heated to about 220°C. with caustic alkalies yields various fatty acids were sufficient to confirm its relation *in vitro* to both carbohydrate and fatty acid (Hoppe-Seyler, 1879; Raper, 1905). The manner in which fatty acids may be formed is by way of the aldol condensation, two molecules of acetic aldehyde yielding aldol (β-hydroxybutyric aldehyde).

$$\underset{acetaldehyde}{2CH_3.COH} \longrightarrow \underset{aldol}{CH_3.CHOH.CH_2.COH}$$

The aldol condensation, when repeated, might be expected to yield the longer-chain fatty acids. The practical objection that when aliphatic aldehydes condense with acetaldehyde they yield branched-chain compounds has been answered by Raper (1907a), who showed that aldol may be made to condense with itself to form a straight-chain compound, and by Smedley (1911), who showed that this is also true of crotonaldehyde. Biological evidence that fatty acids may be formed in this way is furnished through the butyric acid fermentation of lactic acid by certain

anerobic bacteria, in which not only butyric acid but small amounts of higher acids, octoic and decoic, are formed (Raper, 1907b; Neuberg and Arinstein, 1921). Other evidence as to the biological possibility of such a synthesis is supplied by Friedmann's (1908) experiments, in which he showed that aldehyde ammonia, on perfusion through the surviving liver, gives rise to acetoacetic acid and acetone, which are also formed from aldol in the same way. Reichel and Schmid (1939) working with yeast, *Endomyces vernalis*, gave the following as their conception of the formation of the higher fatty acids from acetaldehyde and other aldehydes:

$$\underset{\textit{acetaldehyde}}{2CH_3COH} \longrightarrow \underset{\textit{crotonaldehyde}}{CH_3.CH{=}CH\ COH} + CH_3COH \longrightarrow \underset{\textit{hexenedial}}{CH_3.CH{=}CH.CH{=}CH.COH}$$

$$\text{(a)}\ 3CH_3.CH{=}CH\ CH{=}CH.COH \longrightarrow \underset{\textit{octadecaoctaenoic acid}}{CH_3(CH{=}CH)_8COOH}$$

$$\text{(b)}\ 3CH_3\ CH{=}CH.CH{=}CH\ COH \xrightarrow{H_2} 3CH_3.CH_2.CH_2.CH{=}CH.COH \longrightarrow CH_3(CH_2CH_2)_3.(CH{=}CH)_5COOH$$

$$\text{(c)}\ CH_3.CH_2.CH_2\ CH{=}CH\ COH + CH_3\ CH{=}CH\ CH{=}CH\ COH + CH_3CH_2.CH_2.CH{=}CH.COH$$

$$\longrightarrow \text{Acid abc} \xrightarrow{H_2} \text{Oleic acid} \xrightarrow{H_2} \text{Stearic acid}$$

Leathes (1908), experimenting with minced pig's liver, found that the amount of fatty acid increased after incubation for 24 to 48 hours at 37°C. Addition of various substances likely to be intermediate products in the change (glycogen, glucose, etc.) had no effect, so that, except for involving the liver in the later stages of the process, these experiments tell nothing about the process.

Lubrzynska and Smedley (1913) offered another hypothesis, involving pyruvic acid, which is also a hypothesis of the decomposition of dextrose. Their conception involves the following steps:

(1) The change of pyruvic acid to acetaldehyde by loss of carbon dioxide.

(2) The condensation of acetaldehyde with pyruvic acid forming a keto acid which, by loss of carbon dioxide, forms crotonic aldehyde.

(3) The condensation of crotonaldehyde with pyruvic acid to form a higher keto acid.

The processes, when repeated, should form even-numbered carbon chains convertible into the corresponding acids.

These hypotheses are suggestive, but all are alike in that they encounter difficulties, both chemical and physiological, when the attempt is made to carry the processes up to the actual naturally occurring fatty acids, difficulties which have not yet been overcome. In all these suggested syntheses an important probability has been noted by Pearson and Raper (1927) that compounds of a considerable degree of unsaturation would be formed as stages in the synthesis. The relation of these

compounds to the occurrence of unsaturation in the naturally occurring fatty acids is obvious.

The fact that those fatty acids which are multiples of six carbon atoms (C_{18} and C_{24}) are quantitatively the most important of the naturally occurring fatty acids led Emil Fischer years ago to suggest that these acids were formed by condensation of hexose molecules, but his suggestion lacks chemical and physiological support although it continues to stimulate interest. Klenk (1929) has recently drawn attention to the fact that most of the lipid constituents of brain contain C_{18} and C_{24} acids, and that galactose, cerebrosides, sphingosine, etc., are compounds containing multiples of six carbon atoms. It has also recently been shown that the food of the brain is probably lactic acid, of which the relationship to the hexoses is well recognized (Himwich, 1932).

The rate and extent of the change from carbohydrate to fat is shown in the following experiments. Bürger and Rückert (1931) fed 150 grams of glucose to a fasting goat during a period of hourly milkings and found a sixfold increase in milk fat at the next milking. In a young pig, Wierzuchowski and Ling (1925) found that more than half of the ingested carbohydrate was changed to fat. The production of fat amounted to about 2 mg. per second, and at times 125 grams per day or 0.9 per cent of the animal's weight. After feeding starch, the respiratory quotient was maintained at 1.40 for at least 20 hours and a maximum quotient of 1.58 was attained, which is the highest ever recorded.

The possible effect of insulin on fat synthesis in the liver has been reviewed and reinvestigated by Raper and Smith (1925). Since insulin reduces lipemia and acetonuria, it might be expected to have an effect on fat metabolism; but after insulin, they could find no analytical evidence of fat synthesis in the liver in decerebrate cats. In mice they found, in contrast to the results of Terroine and Weill (1913), that the fat in starved animals and in insulin-treated and starved animals was so variable that no conclusions could be drawn. It seems likely that whatever effect insulin has would be exerted through its effect on carbohydrate metabolism and specifically through its effect on liver glycogen. Although insulin ordinarily brings about a discharge of liver glycogen, it may cause glycogen deposition when the amount already present is small. The presence of glycogen in the liver would inhibit the ketone body formation from fat and hence bring about a lowering of these substances in the blood.

Other factors concerned in the formation of stored fat are discussed in the work of Hilditch and Pedelty (1940), who regard the adipose tissues as dynamic rather than static reservoirs in that there may be not only rearrangements of the fatty acids with formation of new molecules

of triglycerides but also changes in the fatty acid molecules themselves. These interconversions have been demonstrated using fatty acids labeled with deuterium by Stetten and Schoenheimer (1940). Feeding either labeled stearic or palmitic acids, they gave the following scheme to illustrate the changes which take place:

$$\begin{array}{ccccc} \text{Stearic} & \rightleftharpoons & \text{Palmitic} & \rightleftharpoons & \text{Shorter-chain acids} \\ \updownarrow & & \updownarrow & & \\ \text{Oleic} & & \text{Palmitoleic} & & \end{array}$$

They also found that cetyl alcohol is interchangeable with palmitic acid and with stearic acid, as would be expected from this diagram.

Synthesis of Sterols

Evidence for the synthesis of cholesterol in animals and of other sterols in plants has been sought by many workers. Some of the results have been reviewed by Randles and Knudson (1925) and by Pfeiffer (1930), and other representative work is reported below.

Ellis and Gardner (1909), from a comparison of the content of eggs and of newly hatched chickens, concluded that there was no synthesis of cholesterol during incubation of hens' eggs. Beumer (1925) also found no evidence of a synthesis in hens' eggs during the development of the chick. On the other hand, Thannhauser and Schaber (1923) and Dam (1928) reported the opposite results from similar experiments. Gardner and Lander (1914) found no evidence of cholesterol synthesis in chickens fed on a diet extracted with fat solvents. Kusui (1929) studied the cholesterol in incubating eggs from three to twenty days old, using the digitonin method for determination, and found that free cholesterol decreased steadily from 0.273 to 0.119 gram, while cholesterol esters increased from 0.031 to 0.508 gram. There was a decrease of total cholesterol up to the fourteenth day of 0.304 to 0.227 gram, then an increase reaching 0.272 gram on the twentieth day, from which he concluded that the chick embryo in its later stages is able to synthesize cholesterol. Dam (1931), in his study of the changes in cholesterol in growing chickens, found that in the first two weeks there was a decrease in total cholesterol, but in two months there was an increase.

Dezani (1913) reported that white mice given cholesterol-free food showed distinct undernourishment compared with mice fed on an unextracted diet. In later experiments with white rats, Dezani and Cattoretti (1915) succeeded in keeping animals for ten weeks on extracted corn meal and casein. These animals gave evidence of a cholesterol synthesis since their cholesterol content was roughly double that of controls killed at the beginning of the experiment. Randles and Knudson (1925) give experimental evidence to show that the organism

of the white rat is able to synthesize cholesterol. Channon (1925) fed rats on a cholesterol-free diet and found a gain of from 100 to 220 mg. of cholesterol from weaning time up to 100 grams weight; 100-gram animals synthesized 240 mg. per animal in six weeks. Eckstein (1938) found that rats on a high-fat diet (32 per cent) synthesized more sterol than on a low-fat diet, and more on corn oil (iodine number 118) than on coconut oil (iodine number 8). There was a negative cholesterol balance of 9.4 mg. per day on 28 per cent fat and 2.7 mg. on 5 per cent fat. The negative balance decreased with age but was not affected by sex. The hair sterols were not affected. In starvation in the mouse, cholesterol appeared to increase, but the fact was that its amount actually did not change much, the increase being due to the general loss of weight (Valla, 1935).

In contrast to earlier work on chickens, Gardner and Fox (1921), from experiments on human subjects fed over ten-day periods, concluded that "there must be some organ in the body capable of synthesizing cholesterol." From subsequent work (1922) in which they allowed the livers and spleens of man and ox to autolyze at 37°C. and found no change in the total sterol content, they concluded that neither organ had any part in the synthesis or destruction of cholesterol.

Gamble and Blackfan (1920) compared the cholesterol intake with the excretion in infants over three-day periods. They found in all experiments a much larger excretion than intake and interpreted the result as indicating a synthesis of cholesterol. This evidence would have been more conclusive, however, if the experiments had been continued over a longer time. Beumer and Lehmann (1923) reported experiments upon newborn puppies which were fed for four weeks on a diet low in cholesterol. They found an increase in that time amounting to twenty times the amount of cholesterol furnished in the diet. They inferred, therefore, that cholesterol was synthesized from substances not found in the ether extract of the food. Later, however, Beumer (1925) found that the total cholesterol of puppies which had been starved for seventeen days was the same as at the beginning of the starvation, taking account of the fecal sterols. There was therefore no basis for assuming either a synthesis or a destruction of cholesterol. Fox and Gardner (1925) reported that in infants during the colostrum period the excretion of cholesterol exceeded the intake and that therefore there must be new formation. In normal adult men (26 cases) the average intake of cholesterol (precipitable by digitonin) was 0.253 gram and the output 0.56 gram, a negative balance of 0.307 gram which must have been synthesized. Bürger and Winterseel (1929) studied the sterol balance in a human being with a closed bile duct on a diet of bananas and bread and butter, and obtained

the following figures: food sterols 0.367 gram, total cholesterol excretion 0.979 gram, excess 0.612 gram. There was therefore an undoubted synthesis, a finding that gains further weight from the fact that plant sterols are not appreciably changed into cholesterol in the animal body (Schoenheimer, 1929). Pfeiffer's (1930) work on calves indicated that a synthesis of cholesterol was possible but improbable. Schoenheimer and Breusch (1933) give an excellent picture of the behavior of the animal body toward cholesterol: synthesis when cholesterol is not supplied with the food, and destruction when it is supplied in excessive amounts. They found that mice on a diet of bread synthesized as much cholesterol in one month as their bodies originally contained. Addition of increasing amounts of cholesterol resulted in decreasing synthesis until, when enough was given, there was no synthesis but considerable destruction. Fat or carotene had no effect on the cholesterol balance. Bile salts increased the destruction, probably because of better absorption.

Schoenheimer's (Rittenberg and Schoenheimer, 1937) work with cholesterol and heavy hydrogen led him to the conclusion that cholesterol was not formed from any steroid in the food or by cyclization of the fatty acids as such, and he suggested rather that cholesterol was formed by the coupling of smaller molecules, possibly those which have been postulated to be intermediates in the fat and carbohydrate metabolism.

Very little information is available as to sterol synthesis in plants, but, since sterols are universal plant constituents, their synthesis may be taken for granted. Beumer (1933) found that sterol activity proceeded actively during germination and growth.

In spite of occasional contradictory evidence, therefore, there is no doubt but that the animal as well as the plant can synthesize sterols. Whether the synthesis is extensive enough for its optimum needs has not yet been shown, although such seems to be the case. In fact the animal body seems to suffer more from excess of sterols which it cannot get rid of (gall stones, sterol deposits in xanthomatosis, arteriosclerosis, etc.) rather than from too little.

Synthesis of Phospholipids

Until very recently, there was little direct evidence of the synthesis of the phospholipids in the living organisms. It was assumed that they must be synthesized as needed for body growth or the hypertrophy of parts, for example, muscle. It was known that they increased in the embryo during the incubation of hens' eggs. They increased in the blood during the absorption of fat, but the increases could not be regarded as positive evidence of synthesis, since they may have been merely in transport from other places. Experimenters, such as Leathes, assumed as part

of their theories of fat metabolism that phospholipids were synthesized, mainly by the liver, but there was little evidence to support their assumptions. London (1928) and his associates furnished direct evidence of the synthesis of phospholipid by the liver, which they obtained by taking samples of blood before it entered and after it left the liver, making use of his ingenious method of entering the vessels as desired by means of canulae attached to the vessels and protruding outside the animal's body. Recently, there has been an accumulation of new facts which leaves little doubt that the intestinal mucosa and the liver are the sites of extensive phospholipid formation. The evidence consists of experiments with labeled fatty acids (Sinclair, 1935) and with labeled (radioactive) phosphorus (Hahn and Hevesy, 1938; Artom, Sarzana and Segré, 1938; and Haven, 1939) by means of which it has been possible to show that in both intestinal mucosa and liver, and possibly the kidney, the labeled fatty acids or phosphorus appear promptly in the phospholipids of these tissues in large amounts. The synthesized phospholipid is promptly removed from these organs by the blood and carried to the other tissues. In the same way, it has been shown that labeled fatty acids and radioactive phosphorus appear in the phospholipids of most other tissues; but their appearance is much slower, and the inference is that the phospholipids formed in the intestine and liver replace the phospholipids of these tissues which do not synthesize them. The brain is one of the organs in which the turnover of phospholipid in the adult is very slow. Apparently the brain phospholipid is formed very early in life and changes very little afterward. Fries, Changus and Chaikoff (1940) found in rats that the highest phospholipid activity was present on the day of birth in all parts of the central nervous system, while at 50 grams' weight the turnover was only 5 per cent of what it was the first day. After the rat reached a weight of 50 grams, it continued to diminish. The phospholipid activity was highest in the spinal chord in a rat up to 50 grams in weight, after which the activities of the parts of the brain were equal or greater. Detailed discussion of phospholipid synthesis will be found in Chapter II and in this chapter under "Fat Deposition and Mobilization."

Intermediary Metabolism and the Role of the Liver

Fat Deposition and Mobilization

Mobilization and storage in the liver

Visible fatty changes in the liver have long been known to pathologists, and speculation as to the cause has ranged from degeneration of the protein to form fat—hence the term "fatty degeneration"—to fatty

infiltration or mobilization. When it was shown by chemical analysis that in many cases of fatty degeneration the total fat content of the fatty liver was often not greater than normal, the conception of fatty degeneration gave way to another: that the fatty material which is normally invisible becomes visible as the result of abnormal conditions. More recent investigations have introduced other conceptions, among them the glycogen-fat balance of Rosenfeld (1929). Thus in phosphorus poisoning there is no mobilization of fat to the liver if the liver contains glycogen, but, as Ray, McDermott and Lusk (1899) express it, when the glycogen disappears or is absent, fat moves into the sugar-hungry cells. Shibata (1911) found in mice that not only was there no increase of fat in the liver after phosphorus poisoning when the liver has a good store of glycogen, but there was a considerable decrease. These facts should not, however, be taken too literally in implying that there is any constant relation between the glycogen content of the liver and its fat content. For example, in diabetic coma, Geelmuyden (1920b) found that the glycogen content of the liver varied from 0.96 to 3.03 per cent, the fat from 2.63 to 7.32.

Hartley and Mavrogordato (1908), as the result of studies on fatty human livers, came to the conclusion that the excess fat found was fat from the depots transported to the liver. Imrie (1914) in Leathes' laboratory, working with fatty livers in various conditions in humans using for comparison the heart and kidney lipids in the same subjects, came to the conclusion that all increases in total fatty acids in the liver over the normal 3 per cent were due to fat transported from the depots. Goldberg (1923) found in livers in tuberculosis that the greater the percentage of fat, the nearer its iodine value approached that of the adipose tissue fat, and that therefore the excess fat of the liver was depot fat.

These conceptions all involve the assumption that the liver acts as a temporary fat store. Terroine (1920), as the result of analyses of the fat content of tissues of animals in all stages of inanition and superalimentation, concluded that the liver in the normal animal is, like the kidney, not a fat store. In his experiments on the dog and rabbit, the liver was the organ which varied least in its total fatty acid content, whether the animal was fasted, overfed, or normally fed. Only in young pigeons and geese was it possible to produce any considerable storage of fat. Leathes and Raper (1925) have expressed their opinion of the behavior of the liver as a fat store in the statement that normally the liver keeps up with its work (of desaturation) so that there is ordinarily no accumulation of fat there. The conclusion was drawn that the liver is a workshop for fat metabolism and that fat storage takes place there only when the fat arrives from the intestine or the stores faster than it

can be worked up. Normally, the accumulation is short-lasting and the liver is to be regarded as a way station in fat transport rather than as a depot.

In hibernating marmots, Mayer and Schaeffer (1914) obtained results which indicated that the fat of the liver increased during the earlier period of hibernation, decreasing later. Berg (1924), working with hibernating salamanders, found that the fat of the liver diminished slowly up to March, then increased at the expense of the fat of the muscles. These differences may probably be explained by differences in the rate of mobilization of stored fat.

Munk early called attention to the accumulation of fat in the liver during fat absorption, and his observations have been shown to be correct by many workers since. Among the earlier work on the role of the liver in fat metabolism is that of Paton (1896) who found the following: The amount of fatty material is uniform throughout the liver. Phospholipid averages about 2.35 per cent of the moist weight and contains about half the liver fatty acids. Fats may be transported to and accumulated in the liver and may also be formed there, but excessive fat is usually gotten rid of in six to eight hours.

Joannovics and Pick (1910) found a great increase of fat in the liver after fat feeding and also that the fatty acids had a higher iodine number than those of the fat fed. This high value decreased rapidly and in twenty-four hours was practically normal again, indicating removal of the accumulated fat. The establishment of an Eck fistula prevented the accumulation and showed that the portal vein was important in the process. Bile or pancreatic fistulas prevented the accumulation, probably because of the slowing of the entry of fat into the blood, which rendered unnecessary the emergency storage in the liver. The additional important finding, foreshadowing later work on phosphorylation of fat by the liver, was that the liver phosphatides, while not increased in amount, had a higher iodine value than before the feeding. This led them to conclude that the phosphatides take up the unsaturated acids for the purpose of bringing about a saturation, the liver acting as a regulator of the degree of unsaturation of the body fat. Iodized fat was not taken up by the liver lecithin. After very large doses of fat, Artom (1933) found that the phospholipids of the liver increased definitely, up to 35 per cent at the fifth to eighth hour, and then decreased rapidly. The neutral fat increased, but the increase was often small. The increase in phospholipid was considered favorable to the idea of a participation of phospholipid in fat metabolism, a conception which was borne out in later work by Artom and others (see p. 294).

As discussed earlier, Leathes decided that these large migrations of

fat resulting in fatty livers were exaggerations of a normal process. The fat normally moved to the liver to undergo the first stage in its oxidation, which he and his associates (1909a,b) believed was a dehydrogenation or desaturation because the fatty acids in the liver had a higher iodine number than that of the stored fat.

As already mentioned, considerable emphasis has always been placed on the glycogen-fat balance in the liver as a factor in fat mobilization, fat accumulating only when the glycogen content was low. Directly connected with the carbohydrate-fat balance in the liver are the effects produced by the endocrine secretions. Coope and Chamberlain (1925) found that injection of pituitrin caused a doubling of the fat content of the liver, an effect which disappeared in about thirty hours, a result in agreement with the work of Coope and Mottram (1914) that increased pituitary action in late pregnancy produced an increase of fat in the liver. In Fröhlich's syndrome (a condition in which pituitary secretion is lacking) the depot fat increased enormously due to the failure of mobilization (Coope and Chamberlain, 1925). Coope (1925) found that insulin inhibited the action of pituitrin. Hynd and Rotter (1932), experimenting with pituitrin and pitressin, produced an infiltration of fat into the liver accompanied by a decrease of liver carbohydrate in the first few hours, a result which was later followed by the reverse change. On muscle glycogen, the effect was just the opposite. Pituitrin, being a mixture, was less effective than pitressin, and pitocin was antagonistic to pitressin in its effect on liver glycogen and fat. Burn and Ling (1930) found that the anterior pituitary hormone increased ketone body output in rats, a finding which has been abundantly confirmed both for rats and also for other animals. It also brings about increases in liver fat (Best and Campbell, 1936) and in blood ketones (Mirsky, 1936). Since ketone bodies are produced from fat and almost exclusively in the liver (Blixenkrone-Møller, 1938c), it is probable that one purpose of fat mobilization to the liver is the formation of ketone bodies which are then burned in other tissues. Whether the hormone (or hormones) acts specifically on fat metabolism or whether it acts indirectly by suppression of carbohydrate metabolism is uncertain. The experiments of Shipley and Long (1938) negate the occurrence of a special enzyme for fat metabolism and indicate that the ketogenesis is an indirect result of the suppression of the carbohydrate and especially of the protein metabolism by the anterior pituitary hormone. Specific information on the anterior pituitary hormone has been submitted by Greaves, Freiberg and Johns (1940). Extraction of the glands at pH 11 gives the highest yield. The hormone is heat-labile, non-diffusible, and salted out by full saturation with sodium chloride or 0.2-0.45 saturation with ammonium sulfate. Its iso-

electric point is pH 6.7-5.75, but it is more stable at pH 9.5-11. The unit is defined as the amount which will reduce the respiratory quotient in rats to 0.80 (from 0.86-0.94) and contains 0.17 mg. protein. The best preparations are rich in the growth factor. Ketogenic power and R. Q.-lowering run parallel.

Along with this hormonal regulation of fat transport may be considered the nervous regulation. Wertheimer (1926) showed that section of the upper thoracic portion of the spinal cord or of the liver nerves prevented the accumulation of fat in the liver in phlorizin poisoning because it prevented its mobilization from the depots. Section below the seventh thoracic did not alter fat mobilization. He found also (1931) that insulin inhibited fat mobilization in phlorizinized dogs and that large doses of epinephrine interfered with fat mobilization. Hepner and Wagner (1927) found that insulin inhibits desaturation of fatty acids by the liver (probably by inhibiting mobilization).

The effect on the blood of mobilization of fat to the liver either from the intestine during absorption or from the stores has been considered in Chapter III. In general, there is always some free fat in the blood in the form of minute globules (chylomicrons). In the postabsorptive state the amount of free fat is at a minimum but may increase to an amount which is visible as a milkiness during fat absorption and occasionally in fasting. In fasting, the hunger stimulus may be great enough to cause the discharge of fat into the blood from the stores at a rate greater than the liver can take it up, especially if there is a difficulty in its combustion as happens in severe diabetes. In this case and probably in others, as for example in the fatty livers of depancreatized dogs, there is a great accumulation in the liver both of fat and of some of its metabolic products such as cholesterol esters. One result of this accumulation is a greatly enlarged liver. In some cases, as in the enlarged liver and spleen observed by Holt, Aylward and Timbus (1937) in idiopathic lipemia, other organs associated with the liver in the reticuloendothelial system, as for example the spleen, enlarge also. Probably the phenomena of lipid accumulations in Gaucher's disease, the Schüler-Christian disease, and the various types of xanthomatosis are produced in much the same way. Due to some interference in utilization, the various lipid products which have a limited path of excretion from the body are excreted "interiorly."

A great deal of experimental data on the factors producing fatty livers has accumulated in the last few years. Blatherwick and associates (1931) reported a substance in beef liver which causes a great deposition of fat and cholesterol in the livers of rats on a generous diet high in fat and low in carbohydrate. Values of cholesterol up to 5.5 per cent

and total fatty acid up to 11.5 per cent were obtained. Phospholipid values were unchanged. The active substance was soluble in water and boiling alcohol. Best and associates (1932) observed that depancreatized dogs could be kept in satisfactory condition for a long time with insulin but would eventually fail and die, and a characteristic feature was the presence of very fatty livers. The fatty livers could be prevented by feeding raw pancreas, lecithin, or its constituent, choline (or by betaine), or by food materials which contain or yield choline. One way in which choline acts has been shown by Welch and Welch (1938), who found that when arsenocholine was fed it could be demonstrated in the liver lecithin to the extent of 2 per cent. Perlman and Chaikoff (1939), with the help of radioactive phosphates, were able to show that choline speeds up phospholipid formation. With choline, there was increased formation and increased removal of phospholipid lasting for ten hours. The increase was proportional to the dose of choline. Best and associates (1939) reported that choline would prevent and cure the fatty livers produced by carbon tetrachloride poisoning. Griffith and Wade (1939) found that a deficiency of dietary choline in young rats resulted in a very toxic state with hemorrhagic degeneration of the kidneys. The abnormality was prevented by an amount of choline too small to affect liver fat. These results indicate that choline may be a limiting factor in fat removal from the liver.

Various other factors in feeding which influence fatty livers have been investigated. Hortenstine, Chanutin and Ludewig (1938) found that normal rats developed fatty livers on a diet high in yeast (20-80 per cent) and that the extent of the fat accumulation varied with the amount of yeast. Partially nephrectomized animals were not affected. Tucker and Eckstein (1938) and Channon, Manifold and Platt (1938) found that methionine inhibited the accumulation of fat in the liver while cystine had the opposite effect. Best, Grant and Ridout (1936) found that a diet containing casein 10 per cent, gelatin 20 per cent, and fat 40 per cent produced fatty livers, but that 30 per cent casein and no gelatin did not, which may be because of the lack of methionine in the gelatin.

Lipocaic (an extract of pancreas) was active as an inhibitor to the extent of about three times its choline content (Channon, Loach and Tristram, 1938). In insulin-treated depancreatized dogs, Dragstedt and associates (1939) found that the blood lipids rose during the first week and then declined to about half the normal level, the decline being accompanied by a fatty infiltration of the liver. Oral administration of lipocaic corrected the hypolipemia and maintained the blood fat at the normal level. Norris and Donaldson (1940) found that an alcoholic extract of beef liver contained a substance essential for the growth of

young salmon. It was none of the known vitamins. An alcoholic extract of beef pancreas increased the rate of growth and ultimate size.

The origin of the liver fat was studied by Barrett, Best and Ridout (1938) by the use of deuterium. In fasting, after pituitary extracts and after carbon tetrachloride poisoning, the fat was depot fat. On high protein or high carbohydrate, the fat was not depot fat but probably food fat.

Vitamin B_6 deficiency was found by Halliday (1938) to produce significantly higher liver fat than in normal animals. Choline reduced the liver fat in the B_6-deficient animals but never brought them down to normal levels.

Phospholipids of the liver in fat metabolism

The idea that the phospholipids ("lecithin") take part as intermediate stages in fat metabolism dates back many years. Loew (1891) believed that the fat was changed into lecithin in order to pass into the tissue cells, lecithin being the soluble and transportable form of the fats. He believed that the lecithin form was necessary also for oxidation, lecithin acting as a machine for burning the fatty acids. Work of Thunberg (1911) on oxidation of the fatty acids puts special emphasis on the lecithin form of combination, especially in the presence of traces of iron.

Mayer and Schaeffer and Terroine have shown that the percentage of phospholipid in liver, like that in other parenchymatous organs, is ordinarily quite constant under a variety of conditions, a conclusion supported by the more recent work of Bloor (1928) on normal beef livers and that of Sinclair (1929) on cat livers during fat absorption and, in general, by modern workers. Mayer and Schaeffer (1914) showed that the percentage of phospholipids may be greatly increased in the livers of dogs and rabbits by lowering or raising their body temperature, and in guinea pigs and rabbits by diphtheria toxin. Heffter (1891) found that the lecithin content of the liver was lowered about 50 per cent by phosphorus poisoning and slightly by hunger. Theis (1928b) found it possible to lower the percentage of liver phospholipids in rabbit livers by insulin, the lowering being especially marked at the time when the effect of insulin was greatest. He had previously noted (Theis, 1928a) in an abnormal beef liver that the percentage of phospholipid was markedly below normal, while the neutral fat was high.

Artom (1933), in discussing other conditions which affect the phospholipid content of the liver—thyroidectomy, pancreatectomy, and ovariectomy—gave the results of a series of experiments in which, after feeding large amounts of fat (20-35 grams per kilo), the phospholipid content

of the liver was notably increased—up to 35 per cent at the seventh hour after feeding. This increase during fat absorption Artom regards as evidence that the phospholipids are normal stages in the intermediary metabolism of neutral fat.

Thus, while the thesis of Mayer and Schaeffer of a characteristic constancy in phospholipid percentage of tissues in the normal state may in general be accepted for the liver, the possibility must be admitted that the percentage content may be changed under unusual conditions.

That the nature of the combined fatty acids may normally respond to changes in food fat, rapidly in the case of organs directly connected with absorption such as the intestinal mucosa and the liver, and more slowly in the case of other organs and tissues, has been shown by Sinclair and others. Especially in the case of the liver is there the probability of the fatty acids of the transported fat, either from the food or from the fat stores, becoming temporarily a part of the liver phospholipid.

Table 41, taken from Sinclair's work (1929), indicates what may be expected as to acute and longer-continued changes in the liver phospholipids as the result of food.

Table 41. Comparative Effect of Absorbed Fat on Phospholipid Fatty Acids of Liver (Sinclair, 1929).

Cat No.	Time of Feeding Before Death (hours)	Weight in Moist Tissue (%)	Weight in Dry Tissue (%)	Iodine No.
		Control Cats*		
4	24	1.65	6.87	105
6	24	2 08	7.98	120
9	48	2.50	11.50	120
Av.		2 08	8.78	115
	Cats fasted 24 hours and then fed 13 to 14 grams of cod liver oil*			
2	2	1.99	8.74	115
3	4	2.38	11.44	131
5	6	2.23	10.23	134
7	8	1.98	8.50	140
8	24	2.04	10.72	159
10	46	2.48	12.08	159
13	75	2.58	11.32	129
11†	9	1.86	7.68	110
12‡		2.48	11.00	142
Av.		2.22	10.19	

* All cats in this series were fed on a standard diet of 3 parts of boiled lean beef (the fat which rose to the surface was removed) and 1 part of bread for at least 2 weeks before use.
† Fed olive oil.
‡ Fed cod liver oil at 48 hours, and olive oil at 7 hours before death.

Sinclair (1936) later found that the fatty acids of the phospholipid of the blood also changed in the same direction under the influence of the fat of the food. In investigations (1931) on young rats which were made to double their weight (a) on a fat-free synthetic diet, and (b) on a diet containing considerable cod liver oil, he found that the fatty acids of the phospholipid of the whole animal had an iodine number of

101 on the fat-free diet and 145 on the cod liver oil diet, indicating that the nature of the phospholipid of animal tissue may be greatly changed by diet and also that tissue phospholipid may perform normally with a considerably varied type of unsaturated fatty acids.

Artom, Sarzana and Segré (1938) found that radioactive phosphorus fed to rats on carbohydrate and fat diets caused increased total radioactivity, greatest in the liver and intestine; then in kidney, lungs and spleen; then heart, testicles, and muscles; and least in the nervous tissue. With fat, the difference between tissues was more marked than with carbohydrate. These authors interpret the results to mean that the food fat increases the synthesis of phospholipid in the intestine and liver and the phospholipid is distributed by the blood to the other tissues.

Fries and associates (1938) found that feeding fat increased the turnover of radioactive phosphorus by the liver and intestine.

The change of fat to carbohydrate

The transformation of carbohydrate to fat in animals is generally accepted. It might be expected that a formation of carbohydrate from fat would take place by a reversal of this process, since the reactions taking place in the living organism are often of the reversible type. The change from fat to carbohydrate in the plant kingdom has been shown and the energy changes in the process determined in some cases. For example, in molds grown on a medium containing fatty acid but no carbohydrate, Terroine, Bonnet and Duquenois (1927) have shown that the formation of carbohydrate takes place with an efficiency of 75 to 80 per cent, a value about the same as that found for the formation of cellulose from fat in the germination of seeds. The loss of energy in the transformation is slightly lower for the unsaturated acids than for the saturated, and is less the greater the degree of unsaturation, which indicates that the formation of unsaturated acids is a stage in the formation of carbohydrate from fat in the plant. The evidence regarding the transformation of fat to carbohydrate in animals is large in amount, but is indirect and based on assumptions for which there is often insufficient experimental basis. Nevertheless, there are many, especially in Europe, who believe that the evidence available supports the fact of the transformation.

The problem has been attacked from several angles. Most of the earlier work was done on experimentally diabetic animals on the assumption that any carbohydrate formed in such animals would be excreted as sugar and could be determined in the urine. In using this method, known sources of sugar are estimated and the excess excreted over the calculated yield from these sources is assumed to be derived from fatty

acids. The difficulties involved are obvious. The potential sugar available from the food, including the glycerol of fats, can be estimated, but the amount available from body glycogen is not easily determined. The ability of the diabetic animal to burn sugar is not definitely known. Several experimenters have attempted to get evidence on the formation of sugar from fat by perfusing the isolated surviving liver, since it seemed likely that the liver was involved in the process, while others have investigated the changes in liver glycogen under various conditions. More recently, direct evidence *in vivo* has been obtained by tapping the blood vessels entering and leaving the liver. Entirely different from these methods is one based on a study of the gas exchange. The respiratory quotient obtained when fat is being changed to carbohydrate is lower than when fat is being burned, and respiratory quotients below those for fat combustion are taken as evidence that fat is being changed to sugar; but the difficulties in adequately controlling conditions and in interpreting the results are great, and the conclusions are of doubtful significance.

One of the earlier conceptions regarding the change of fat to carbohydrate, that of Geelmuyden (1921), was based on the idea that the acetone bodies, well-known products of the breakdown of the fatty acids, were the building stones of which carbohydrate was made. In dogs treated with phlorizin, acetoacetic and β-hydroxybutyric acids are formed and appear in the urine together with a large output of sugar; the glycogen vanishes from the liver, and there is an accumulation of fat there. As soon as the animal is fed carbohydrate, these acids disappear from the urine, the fat in the liver disappears, and some glycogen is formed. There is thus a physiological antagonism between the ketonuria and fatty liver on one hand and glycogen storage on the other; for when glycogen disappears from the liver, fat accumulates there, and when glycogen accumulates, fat disappears. Hence, when glycogen vanishes from the liver, the sugar-hungry organism forms sugar from protein and fat. The accumulation of fat in the liver is considered to be for the purpose of producing carbohydrate and in that process the acetone body acids are formed as steps or by-products. Their disappearance on carbohydrate feeding is not the result of combustion but of a change into carbohydrate. The change from fat to carbohydrate takes place more quickly and easily if the liver contains glycogen. If the liver does not contain glycogen, the formation of sugar from fat takes place slowly and fat (mobilized for transformation into carbohydrate) accumulates in the liver. Because of the slow formation of sugar from fat, the phlorizinized animal must form its sugar from protein, which is shown by an enormously increased protein metabolism reaching to five times what it is in simple hunger.

Geelmuyden (1920a) insisted that all changes in metabolism must be taken into account, not only the glycogen, blood sugar, glycosuria, and ketonuria, but the migration of fat, the transformations of protein, the total metabolism, and the transformations of energy. He believed that carbohydrates promote the formation of sugar from fat, and that the output of sugar is not increased by feeding fat unless a certain amount of sugar is given with the fat. Since the experiments which show no carbohydrate formation from fat have been done on a carbohydrate-free dietary basis, this argument is at present without answer. Stöhr (1933a) offered evidence which tends to confirm Geelmuyden's theory. He found, in his experiments with male fasting rats fed sodium acetate, that glycogen formation did not take place in livers with a glycogen content below 0.3-0.4 per cent, but that above this level it did. In female rats (Stöhr, 1933b), he found that there was glycogen formation from *n*-butyric acid in the presence of glucose but none without it. He concluded that formation of carbohydrate from fat is possible.

The conception of a pituitary hormone regulating fatty acid metabolism leads to some curious conclusions in connection with the possible change of fat to carbohydrate, a transformation which seems to be connected with the formation of ketone bodies. It is well known that pancreatectomy causes a breakdown in carbohydrate metabolism, with failure to burn sugar, resulting in its loss to the organism. Removal of the hypophysis abolishes these symptoms (Houssay, 1932). Since removal of the hypophysis also removes the pituitary with its secretions, it stops the mobilization of fat to the liver and the production of acetone bodies. According to Geelmuyden and others, who believe that diabetes is due to an overproduction of sugar, especially from fat, and that the ketone bodies are stages in the transformation, the phenomena resulting from the removal of the hypophysis are the logical result of the lack of hormone which brings about the transformation of fat to sugar.

Chaikoff (1927) found, in depancreatized dogs which had been kept in a normal metabolic state with insulin, that deprivation of insulin resulted in greater sugar and ketone body production in fat animals than in lean ones. The *D:N* ratio was not stable at 2.8:1, but its mean average value depended on the nutritive condition of the animal, mainly on the state of the fat stores. Thus in the same dog (F), the *D:N* ratio was 2.8 when it weighed 4.8 kilos, but was 2.3 to 4.4 when the animal's weight was 5.9 kilos. Much more sugar was excreted when the animals werê fat. The sources of extra sugar were glycogen and fat. Liver glycogen was available; muscle glycogen was assumed not´ to be, but even if it had been there would not have been enough. Glycogen in muscles would support metabolism for not more than two hours. The

respiratory quotient in these animals was 0.70, 0.66, and 0.69. The *D:N* ratio, after making all allowance for glycogen in liver and muscle, was often found to be greater not only than the 2.8 of Minkowski but the 3.65 of Lusk, for example, 5.08. Correcting for glycerol in the fat burned brought it to 4.0. Some sugar was therefore formed from fat, especially in the fat animals. Ketone bodies were higher in the blood in fat than in thin animals, but less was excreted. In this connection may be mentioned the work of Krogh and Lindhard (1920) on the relative energy value of fat and carbohydrate as sources of muscular energy. As the result of their experiments they found that when fat is being burned, about 11 per cent of its energy is wasted as compared with carbohydrate. As part of the working hypothesis developed from their experiments, they believe that when the respiratory quotient is below 0.8, carbohydrate is being formed from fat and provisionally stored, and that a corresponding transformation of carbohydrate to fat takes place when the quotient is above 0.9 per cent. Hawley (1932) concluded from experiments on phlorizinized fat dogs that the respiratory quotients and *D:N* ratios with and without insulin gave no evidence of conversion of fat to carbohydrate.

Chaikoff and Weber (1928) found that when epinephrine was injected into depancreatized dogs from which insulin and food were withheld, the excretion of sugar in the urine may be far greater than can be accounted for by the preformed carbohydrate, protein, and glycerol, and must therefore originate in the fatty acids. However, Bachrach, Bradley and Ivy (1936) and others have repeated this work, using improved methods, and, on the basis of more modern conceptions of the availability of tissue glycogen, have come to the conclusion that it is unnecessary to assume any transformation of fat to carbohydrate in these animals. Moreover, the results of Anderson and Macleod (1930) show that glycogen determinations under these conditions may be greatly in error.

Soskin (1929) found a small positive excretion of sugar after fat feeding in diabetic animals, and later questioned the assumption that the diabetic organism cannot burn carbohydrate (1930). Deuel, Wilson and Milhorat (1927) have demonstrated that the phlorizinized dog can burn dextrose.

Lueg and Flaschenträger (1925) found that dogs and pigs on an exclusive fat diet do not excrete acetone bodies, from which they concluded that whatever glucose was necessary to burn up the acetone bodies was formed from the fat. Bickenbach and Junkersdorf (1928) found that excessive fat feeding after hunger at first produced hypoglycemia, followed by an abrupt rise in blood sugar, indicating that the sugar-regulating mechanism of the liver was not interfered with. On the other hand, Lusk (1901) showed that the feeding of fat to diabetic humans or experi-

mentally diabetic animals did not produce any extra sugar in the urine, and also showed (1908) that work sufficient to double the fat metabolism in phlorizinized animals did not increase the sugar output.

Perfusion experiments by Burn and Marks (1926) indicated that the formation of sugar from fat by the liver was probable. They called attention to work by Embden (1905), in which it was shown that when a surviving liver was perfused with defibrinated blood there was a production of sugar greater than can be accounted for by the disappearance of glycogen. These results were confirmed by Lattes (1909). Burn and Marks used livers of dogs and cats which had been fed for some days on fat and which contained little or no glycogen. Free sugar and diffusible substances, including lactic acid, were removed by a preliminary perfusion with blood, which was discarded. Further perfusion with blood resulted in the addition to the blood of sugar at the rate of 2 to 4 mg. per gram of liver per hour, which was shown not to come from lactic acid and to only a small extent from protein. No measurement of fat was made, because it was large in amount and unevenly distributed in the different lobes. The formation of sugar was not influenced by insulin, adrenalin, or pituitary extracts.*

Jost (1931) also investigated the change of fat to carbohydrate by the surviving liver; he concluded that phospholipid can be changed to sugar, and believed that the change of fat to sugar took place in the liver. Page and Young (1932), however, showed that the increased sugar could be accounted for by the glycerol of the phospholipid.

Greisheimer (1931) investigated the effect of feeding fat on the liver glycogen in rats. She found that, as the percentage of lard in the diet was increased, the liver lipids increased perceptibly but the glycogen very little, although glycogen was readily formed by feeding the stock diet of sucrose, casein, or glycerol. Maignon (1929) was unable to demonstrate any formation of glycogen from fat in the liver or muscles of dogs. Rosenthal (1931) found that fasting mice oxidize β-hydroxybutyric acid as well as normal animals, and that when fed to such animals, it does not increase liver glycogen—a fact which indicates that this fatty acid was not converted to carbohydrate. Eckstein (1933) fed propionic, butyric, valeric, and caproic acids to rats and found that propionic was the only one which increased liver glycogen—a finding which fits in with the earlier work of Ringer (1913) showing that fatty acids form sugar in the

* With regard to the distribution of fat in the different lobes of the liver, Paton (1896) and Raper and Smith (1925) found an even distribution in the livers of decerebrate cats, whereas Dowler and Mottram (1918) and Burn and Marks (1926) found a variation in different lobes after large fat feedings, indicating that incoming fat is not immediately evenly distributed.

diabetic animal in proportion to their power to form propionic acid (by β-oxidation).

Hawley, Johnson, and Murlin (1930) found in experiments on human beings with very high fat diets that there was a depression of the respiratory quotient below that for the burning of fat. Repeating the experiments with a pig on a high-fat diet (fatty acid to glucose ratios up to 6:1) with no acidosis, Hawley and Murlin (1932) found a low quotient following the meal with a subsequent rise above the original level. The low quotients they at first believed supported the contention that carbohydrate was being formed from fat. However, as pointed out in a review by Mitchell (1933), the low quotients obtained by Hawley and Murlin, while corrected for products of incomplete combustion, were obtained postabsorptive and were followed by quotients above the original level, which may be connected with the withdrawal of acid from the circulation during digestion, followed by a return to the blood later. Hawley, Johnson and Murlin (1933), as the result of further experimentation with high-fat diets, explain the low initial R.Q. after the fat feeding and subsequent rise as the result of an uptake of oxygen by the fatty acids without production of carbon dioxide: a dehydrogenation with resulting unsaturated acids, or possibly the β-oxidation preceding combustion.

More positive evidence in favor of the change of fatty acid to sugar is furnished by a group of recent papers. Gemmill and Holmes (1935), experimenting with rats on a mixed and on a butter diet, found that the respiratory quotient of liver slices of rats on a butter diet averaged 0.58, those on a normal diet 0.79, and the glycogen content of the butter livers increased on shaking in Ringer's solution at 37°C. The glycogen content of the livers of the butter-fed rats fell practically to zero on the first day of the diet, then rose to about 1 per cent on the fourth and fifth days of butter feeding. The acetonuria increased to a maximum on the third or fourth day, and then decreased. The lowering of the respiratory quotient and the increase in glycogen was taken to indicate a change of fat to carbohydrate. The possible effect of the glycerol of the butter was, however, not considered. Deuel and associates (1937) suggest that triglycerides which can be stored (higher) yield no glycogen, but that the glycerides of the lower fatty acids yield their glycerol for glycogen. Odd-carbon fatty acid glycerides yield much more glycogen than even-numbered carbon glycerides, and more than is available from the glycerol present, indicating that odd-chain acids are converted to carbohydrate.

Heller (1936), by sampling the blood entering and leaving the liver, showed that glycogenesis occurred in fasting animals and that the sugar must have originated from fat; *D:N* ratios in the blood up to 15 were obtained, and sugar formation from fatty acid up to 36 grams was demon-

strated, all other sources having been accounted for. Haarmann and Schroeder (1938) found that the lactic acid in surviving organs was increased by the addition of β-hydroxybutyric and dihydroxybutyric acids to the perfusion fluids. This increase was not covered by a loss of carbohydrate, and therefore the acids added must have furnished the extra lactic acid which in turn could readily form sugar. They believe the steps in the conversion to be: butyric → crotonic → β-hydroxybutyric → acetoacetic → dihydroxybutyric → dihydroxycrotonic → diketobutyric → methylglyoxal → pyruvic or lactic acid → sugar. Blixenkrone-Møller (1938b) found, on perfusing liver with sodium butyrate, that 20 per cent is changed to ketone bodies and the rest to carbohydrate. The *D:N* quotient may exceed 20. Caprylic acid yields two molecules of ketone bodies. The change of butyrate to sugar is represented as: butyrate → β-hydroxybutyric or succinic acid → fumaric, malic, oxalacetic, and pyruvic → dextrose.

The state of this subject at the time was very well summed up in a paragraph near the end of Mitchell's (1933) critical review as follows: "The sum total of the evidence for the possibility of a conversion of fatty acids to sugar seems to be merely suggestive, certainly far from conclusive. On the other hand, there is no justification for a categorical denial that the conversion is possible. The verdict of 'not proven' is the most logical one to return."

Obesity

Obesity is that state in which the accumulation of reserve fat becomes so extreme that the functions of the organism are interfered with (Gräfe, 1923). It may originate in two ways: (1) from overnourishment—a normal body taking more food than is used up in its activities—or (2) from endogenous, constitutional abnormalities which interfere with normal disposal.

Obesity which is definitely and solely the result of constitutional (endocrine) abnormality is rare and need not be considered in the present review. However, it is very difficult to exclude absolutely the effect of the constitutional or endocrine factor as, for example, more especially in the specific dynamic effect of foodstuffs.

The modern viewpoint may be obtained from the work of Strouse, Wang and Dye (1924a,b) who also give a good review of the literature. In overweight persons they found that obesity is not caused by abnormalities in basal metabolic rate. Certain persons may become obese on food intakes much below the calculated caloric requirements based on those of the average of normal individuals. In these persons, the basal metabolism is normal but the specific dynamic action of protein is de-

cidedly less marked than in the average. In this sense they are more economical in their metabolism than the normal individual. Whether this is the result of a constitutional difference has not been determined. Wang and Strouse (1925) found that obese individuals, after ingestion of protein, tend to derive their energy from carbohydrate, fat utilization being decreased after a meal, while thin and normal weight persons use less carbohydrate than when fasting; this fact provides a reason for the excessive fat storage by the obese.

Brown and Keith (1924), in a series of 14 obese persons, found that the circulating blood and plasma volumes per kilo body weight were smaller than in normal persons. Bauman (1928) found a relatively low heat production after protein feeding in the obese, indicating a more economical metabolism.

Much significant work on the mechanism of obesity, especially in its relation to gain and loss of water which may mask to a considerable extent the changes in fat, has been carried out by Newburgh and Johnston (1930). They are definite in their conclusion that obesity is solely the result of excessive caloric intake.

Changes in the migrating salmon

The story of the changes in the tissues of salmon during their spawning migration is one of wholesale transformations of one tissue material into another in the same animal—muscle and fat into sex products consisting largely of phospholipids and nucleoproteins. The salmon does not take food during the migration. The story was first told by Miescher (1897) about the Rhine salmon. Additional details were added by Greene (1919, 1921) on the Pacific coast salmon (*Oncorhynchus tschawytscha*) and a similar story of the Amur river salmon is told by Pentegov, Mentov and Kurnaev (1928). The following data from Greene's work are significant in the present discussion. During the 700-mile migration to the spawning ground, the muscles first lose their fat (15 per cent down to 2.4 per cent) and phospholipid (1.18 per cent down to 0.44 per cent); then about 30 per cent of their protein which he thinks is stored protein, since the structure of the muscle does not change. The ovaries increase up to ten times their original weight, although their percentage of phospholipid and fat diminishes somewhat during their growth. Fat and phospholipid are much less important as stored food in salmon eggs than in hens' eggs. Pentegov secured specimens of the salmon (*Oncorhynchus keta*) at the mouth of the river, at various places along the course, and at the spawning grounds 1200 miles from the sea. The weight of head, genital organs, skin, and bones increased greatly during the migration. Water content increased from 67 to 85 per cent

in the females and 69 to 86 per cent in the males. The fat content decreased from 9.2 to 0.17 per cent in the males and from 11.3 to 0.5 in the females. The protein content decreased in the males from 21 to 13 per cent and in the females from 21 to 14 per cent. The males lost 77 per cent and females 79 per cent of their reserves of energy. The average daily expenditure of energy in their passage up the river was for males 25,810 calories and for females 28,390 calories per kilo live weight. With starvation, the iodine number of the fat increased and the saponification numbers decreased, indicating a selective retention of the longer-chain fatty acids of a high degree of unsaturation.

Functions of the Lipids in the Body

Fat

Energy production. Fat being the main form in which energy is stored in animals, it is obvious that it must be used by these organisms for their energy needs, notably for muscular work. The scientific demonstration of its use for this purpose is often surprisingly difficult. From the work of Meyerhof and of Hill in determining the mechanism of muscular contraction, it might be concluded that carbohydrate was the only source of energy for muscular contraction, and there is much other work which supports such a conclusion. Winfield (1915) found that several hours' stimulation with the tetanizing current produced no difference in the fat content of frogs' legs, and that therefore fat had not supplied the energy for the muscular work. Furusawa (1925) found that in exercise, carbohydrate is first used, but in long-continued exercise, fat is called upon, and more readily if the individual is on a fat diet. Cuthbertson (1925), as the result of a small number of experiments on prolonged stimulation of muscles, found that in three out of four the fat content was diminished slightly—not over two per cent—while the phospholipid phosphorus was not diminished. There was a definite increase in blood inorganic phosphorus on exercise, probably by washing out. Exercise enough to diminish the fat content did not affect the phospholipid content. Experiments on isolated muscle for the purpose of demonstrating the use of its stored fat for work thus give negative results. The reason probably is that stored fat is not directly used, either near its storage place or elsewhere, but is first carried to the liver, altered, and then redistributed for use, presumably as phospholipid or acetone bodies.

Henderson and Haggard (1925) found that men on the Yale rowing crew burned fat and carbohydrate in about the same proportion during work as during rest. A fasting (18 hours) athlete during rest had a

respiratory quotient of 0.75; during three minutes of rowing, 0.72; during ten minutes' recovery, 0.73. The work done was six calories per minute for a man of 180 pounds, 6 feet 4 inches tall.

Meyerhof and Himwich (1924), experimenting with rats which were made to do muscular work after a period of high fat feeding, found that the respiratory quotient during work was 0.7, corresponding to an exclusive fat combustion; yet the carbohydrate, and especially the glycogen of the muscles, was diminished by the work. The work of Chambers and associates (1932) (studies on exercise and recovery in dogs fasted for 3 to 13 days after pancreatectomy and under similar conditions before operation) lead them to favor the theory that fat is the fuel of exercise in depancreatized dogs. Chaikoff and Macleod (1929) had previously claimed that depancreatized dogs could burn glucose.

Buchwald and Cori (1931), in an attempt to determine whether fat was used in muscular work, compared the lipid content of gastrocnemius muscles of the rat and frog. One muscle of a pair was strongly exercised, the other not. They found in the rat no difference in the lipid content of the exercised and unexercised muscles—cholesterol, phospholipid, and total fatty acids were the same in both. In the summer frog, the fatigued muscle contained about 20 per cent less phospholipid and total fatty acid than the resting muscle. The muscle of the summer frog had a low glycogen content, which may explain the use of lipid in this case. They think that normally the animals use the fat of the circulating blood for muscular work rather than the lipid present in the muscle itself. Koldaev and Hel'man (1937) found that work did not diminish the phospholipid content of muscle. Training was found to increase the content of this substance, as has also been indicated by work from this laboratory (Bloor, 1937b).

Oxidation catalysis. The role of the unsaturated fatty acids as oxidases or oxygen transport mechanisms, especially in the phospholipid form, was suggested by Fränkel and Dimitz (1909). Meyerhof (1923) found that under the influence of the —SH group the polyunsaturated acids in phospholipid combination took up oxygen to form peroxides. Von Szent-Györgyi (1924) found that the compound formed with oxygen was not a peroxide but an ethylene oxide. Tait and King (1936) found that the fatty acids in phospholipid combination were more easily oxidized than when free. Bloor (1937a) found that phospholipids oxidized by exposure to air could restore the color to reduced methylene blue under bodily conditions. Later work showed that the oxidized phospholipid could not oxidize glucose under bodily conditions either directly or by transference of atmospheric oxygen.

Phospholipids

In the last few years much work has been done on the phospholipids in their relation to fatty acid metabolism and, although much remains uncertain, some facts begin to stand out.

1. Phospholipids play an important part in the absorption of fat from the intestine. A considerable proportion of the fat passes through the phospholipid form during passage through the epithelial cells. It cannot be said at present how much of the fat is so transformed and it may be that this phosphorylation is only for the purpose of accelerating absorption, just as it is for some sugars (dextrose).

2. The liver changes fat to phospholipid whether brought to it from the intestine during fat absorption or by mobilization from the depots. The liver phospholipid changes in nature (corresponding to the absorbed or mobilized fat) and may increase in percentage temporarily if the income of fat is excessive.

3. Phospholipid when fed prevents and cures the fatty livers produced by various conditions—pancreatectomy, cholesterol feeding, or high fat with low protein diet. Choline is equally effective, so that the curative effect is probably due to this constituent of the phospholipid. The mechanism of choline activity is not certain, but it seems to be a limiting factor in phospholipid formation.

4. Since phospholipid increases in the blood during fat absorption, it is probably important in fat transport, especially since the fatty acids in the phospholipid are to a considerable extent the fatty acids then being absorbed from the intestine.

5. It has been shown, especially in organs of intermittent activity (corpus luteum, mammary gland, etc.), that increase in activity is accompanied by an increase in phospholipid, which connects phospholipid with cellular activity. Cholesterol increases also, but to a much less extent. The phospholipid content of muscles is apparently related to the amount of work which they are called on to do.

6. By reason of their content of unsaturated acids (generally two-thirds or more of the acids of the phospholipid is unsaturated), they may take part in the oxidation systems of the organism.

7. In the tissues, they may be either structural or functional elements in the cells, in which case they would change composition relatively slowly, or food material to be burned, in which case they would show a quick response in composition to ingested fat. In most tissues the response is slow.

8. In the developing chick, they serve as sources of phosphorus in bone development.

9. They probably serve as sources of phosphoric acid in the neutrality regulation of the blood by the kidney.

Cholesterol

The part played by cholesterol in the living organism is still obscure, although there is abundant evidence that it is a highly important substance. It is one member of a widely distributed family to which the name "sterid" has been given. Its occurrence is confined to animals, but there are closely related compounds found in plants which have supposedly similar functions.

Cholesterol is readily synthesized by animals as needed and any excess is normally excreted. Apparently it cannot readily be burned. As indicated, it is related chemically to a wide variety of substances some of which form natural esters with the fatty acids, but aside from that have little or no relation to the fatty acids in metabolism.

The esters of cholesterol with the fatty acids are apparently important in the life of animals because they are normally present in the blood plasma in a constant relation to the total cholesterol. These esters are formed during the processes of absorption of fat and cholesterol and are apparently destroyed in the tissue cells, since they are normally present there only in small amounts. The fatty acids in combination with cholesterol in blood plasma are the most unsaturated of all the fatty acids in the plasma, which fact indicates a special relationship of cholesterol to the unsaturated acids.

Other functions of cholesterol in the organism are indicated by the findings that cholesterol and the phospholipids exist in fairly constant relations to each other in the blood and tissues. These relations are different for different tissues and sometimes for different kinds of the same tissue. For example, smooth muscle is sharply differentiated from heart and skeletal muscle by the fact that in skeletal and heart muscle the phospholipid-to-cholesterol ratio is about 14 to 1 whereas in smooth muscle it is 5 to 1. The fact that phospholipid is hydrophilic and that cholesterol is hydrophobic suggests a number of ways in which the constant relationship noted, as well as departures from it, would be useful to the living organism; but up to the present very little satisfactory work has been done to explore these possibilities.

Catabolism of the Fats

Oxidation Mechanisms for Neutral Fats

It is generally believed that the glycerol portion of the neutral fat molecule is burned in the same way as the carbohydrates (Hubbard and

Wright, 1922). It is readily formed from carbohydrates and appears quantitatively in the urine as extra sugar when fed to completely diabetic animals. It is therefore calculated as antiketogenic material in ketogenic-antiketogenic balances.

Regarding the manner of combustion of the fatty acids, the view of Leathes, *i.e.*, that the first stage in the oxidation of the fatty acids was a desaturation or dehydrogenation taking place in the liver, had been generally accepted up to the time that Burr and others reported work on a fat deficiency disease curable by a fatty acid which might easily be formed by desaturation of ordinary food fatty acids. Since that time, there has been less emphasis on desaturation as a preliminary stage in fatty acid oxidation. The final stages in the oxidation were believed to take place in accordance with Knoop's β-oxidation theory that the long chains were broken down by successive removal of two-carbon fragments, preceded by an oxidation at the carbon atom in the β position from the carboxyl group. Whether this is the only mechanism for the oxidation of long chains is now open to some question. Evidence is available which goes to show that the long chains may be broken simultaneously at several points in the chain, yielding two-carbon fragments (multiple alternate oxidation of Quastel) or four-carbon fragments (Deuel) which are then completely oxidized by β-oxidation. The formation of four-carbon fragments—the ketone acids—appears to be the function of the liver alone, under the influence of a pituitary hormone, while the final combustion of the fragments can be accomplished by the muscles and other organs. It has been known for a long time (Friedmann) that the liver could also build four-carbon acids from the two-carbon residues. Attack on the fatty acids from both ends has been shown to be possible (Verkade and van der Lee); and, though the fatty acids which show this property best are not the common food acids, the results reveal another possible path of fatty acid breakdown. The importance of the phospholipids as stages in fat metabolism has been emphasized by the finding that the phospholipids are formed in large amount by the intestine and liver from both food and depot fat and distributed by the blood. Therefore the probability exists that the phospholipids represent fatty acids on the way to combustion, perhaps by a still unknown process.

Unsaturation

Desaturation as a stage in fatty acid metabolism. The theory that desaturation is a stage in fatty acid breakdown arose from the following considerations. Mobilization of fat from the fat stores to the liver has been observed under a variety of conditions: poisoning with phosphorus, phlorizin, or chloroform; pregnancy; fasting; or a high-fat diet. The

last three, coming within the normal range of experiences of most animals, justify Leathes' belief that this mobilization is a normal process, and that the tissue and food fats are carried to the liver for a definite metabolic purpose. London (1928), by means of his angiostomy technic, was able to show that in fasting there was a flow of fat from the stores to the liver. It is a well-established fact that the fatty acids of the liver are considerably more unsaturated than those of the fat stores. This applies not only to the fatty acids of the phospholipids but to those of the glycerides of the liver as well (Kennaway and Leathes, 1909; Bloor and Snider, 1930). The more highly unsaturated neutral fat appears to be confined to the liver (Bloor and Snider, 1930). These two facts—the normal flow of fat from the depots to the liver, and the presence in the liver of combined fatty acids of a higher degree of unsaturation than those in the fat depots—seemed to Leathes to justify the conclusion that the depot fat was carried to the liver to be desaturated, and that desaturation was therefore the first stage in the metabolism of the fatty acids.

Actual evidence of desaturation of fat by the liver is not large in amount, and some of it may be interpreted in two ways. Leathes and Meyer-Wedell (1909) reported that after feeding cod liver oil, the livers of rats contained fatty acids of a much higher iodine number than that of the cod liver oil, a fact which they interpreted as evidence that the liver had desaturated the fatty acids of the oil. Joannovics and Pick (1910) obtained the same results on dogs, finding that the neutral fat of the liver had a higher iodine number than that of the fat fed. Sinclair (1929), in feeding experiments on rats, found that although feeding of cod liver oil raised the iodine number of the fatty acids of both the phospholipids and the fats of the liver, the values did not rise above those of the fat fed. Leathes interpreted his results as a desaturation of the food fat by the liver; Joannovics and Pick interpreted theirs as a selection by the liver of the more highly unsaturated acids of the cod liver oil—which they regarded as a protective function, the liver regulating the degree of unsaturation of the fat passing through; Sinclair thought that the change in nature of the fatty acids of the phospholipids of the liver was part of the mechanism of fat absorption, the phospholipids containing the fatty acids of the food fat.

Mottram (1912) found in the plaice just the reverse of desaturation, the fatty acids of the liver having a *lower* iodine number than those of the muscles of the fish or of its food. Henriques and Hansen (1901) found that the main stored fat of the porpoise and dolphin was so very different from that of the liver (consisting largely of esters of isovaleric acid) that any action by the liver in its utilization was out of the ques-

tion. They found also that desaturation by the liver is not supported by data on the conger eel. Hartley (1909) discovered in pigs' liver an isomer of oleic acid with the double bond in a different position from that in the ordinary oleic acid present in pig fat. Since fat is mobilized to the liver prior to oxidation it was reasonable to assume that this new oleic acid was formed in the liver from stearic acid. However, Channon, Irving and Smith (1934) were unable to find this acid in pigs' liver. Raper (1913) fed coconut oil and examined the volatile fatty acids of the liver. He found that they had a higher iodine number than those of the coconut oil fed, which he considered evidence of desaturation by the liver.

Actual desaturation of fatty acids by isolated tissues *in vitro* was observed by Eaves (1910), who mixed an aqueous emulsion of embryonic chicken tissue with egg yolk and incubated for five days at 37°C. under toluene. He observed a marked increase in the iodine number of the yolk fatty acids in the case of the twelve- and nineteen-day embryo, but none in the six-day embryo; from this he concluded that the early embryo cannot desaturate the fatty acids and therefore presumably cannot burn fat.

If the liver does desaturate the fatty acids, is it the only organ or tissue which desaturates? The work of Imrie (1914) indicates that it is the only organ which does so to any considerable extent, and it was found by Kennaway and Leathes (1909), and more recently by Bloor and Snider (1930), to be the only organ which contained notable amounts of glycerides more unsaturated than those of the depots. A significant contribution to the topic was the work of Klenk and Schönebeck (1932), who found that the high degree of unsaturation of the fatty acids in the neutral fat of liver was not due to C_{18} acids at all, as would be expected if they were the result of desaturation of food or depot fatty acids, but rather to longer-chain unsaturated fatty acids, arachidonic (C_{20}), etc.

A serious blow to the theory of desaturation as a step in normal fat metabolism was given by the work of Burr and Burr (1930) on a deficiency disease, which they showed could be cured by a fatty acid (linoleic) which is just one step more unsaturated than the oleic acid always abundantly present in the animal body, a step which should be easily taken if desaturation was a regular process.

Evidence against the liver as an important factor in fat metabolism was that of McMaster and Drury (1927), who found that the extirpation of 90 per cent of the liver did not change the respiratory quotient in fasting rabbits, although the injury was severe enough to cause death in five days from liver insufficiency. They concluded that it is "highly improbable" that the liver possesses any vital function in fat metabolism.

The evidence against desaturation by the liver as a normal stage in

fat metabolism may be summed up as follows: (a) the increase in unsaturation observed in the liver fatty acids during fat absorption may be just as well explained as the result of a selection by the liver of the more highly unsaturated acids of the food or depot fat; (b) it is necessary to supply unsaturated acids in the food in order to avoid the symptoms of deficiency; (c) the highly unsaturated fatty acids of the liver are not the result of desaturating the ordinary food or stored fatty acids; and (d) as far as the metabolism of fat is concerned (shown by the constancy of the R.Q. after liver removal) the organism can apparently get along quite well without the liver. On the other hand, highly unsaturated acids can apparently be formed even in animals on a diet which produces fat deficiency (Sinclair, 1934); and, as will be shown later, the more highly unsaturated fatty acids are apparently very useful even if not strictly essential to the animal. Dehydrogenation (desaturation) is believed to be a normal stage in oxidation of the fatty acids as well as of other substances, although this type of desaturation takes place from the carboxyl end of the fatty acid chain and not in the middle, as in the cases under discussion. It may be that desaturation of the fatty acids takes place with varying degrees of ease depending on position in the carbon chain.

There are some claims that the fatty acids are desaturated during absorption from the intestine (Tangl and Berend, 1930); but although Schoenheimer and Rittenberg (1936), using saturated fatty acids labeled with deuterium, have shown that desaturation by the organism is certain, they are uncertain regarding the intestine as the site.

Hydrogenation or saturation. Hydrogenation or saturation of fatty acids is believed by Banks and Hilditch (1932) to occur in the fat depots of the pig. In the tunney, Lovern (1936) found that as the content of stearic acid increased, the degree of unsaturation in the C_{18} acids fell. He suggested that the increase of stearic was at the expense of the ethylenic acids to preserve an approximately constant content of saturated acids. Hilditch and Longenecker (1937) expressed the same idea in the case of ox depot fats. Increase of stearic acid was balanced by a decrease in oleic acid, keeping the C_{16} : C_{18} ratio constant.

The need of unsaturated fatty acids by the organism. That the more highly unsaturated acids are useful and probably necessary for the animal is clear from the work of Sinclair (1932). He found that the highly unsaturated fatty acids of cod liver oil were selectively taken up by the body phospholipids of rats and tenaciously retained until the iodine number of the phospholipid fatty acids reached a maximum of about 160. Not until then did the depots begin to take up the unsaturated acids. Rats can apparently do quite well for a considerable time with phospholipid fatty acids of an iodine number of 100, but the fact that

they quickly appropriate the acids with a higher degree of unsaturation indicates that these acids are especially useful. This behavior also makes it clear that they cannot manufacture these useful acids for themselves (or cannot manufacture them fast enough), but must be supplied with them in their food. A fact regarding the unsaturated fatty acids which has recently come to light and which is probably of considerable significance in their behavior in the body is that C_{18} acids with the double bonds in the ordinary 9-10 position behave toward lipases and films on water like saturated acids of half their chain length (Balls, Matlack and Tucker, 1937).

Fat deficiency; essential fatty acids. Drummond and Coward (1921) found that young rats, grown from weaning to maturity on diets deprived as far as possible of fat, showed normal development and behavior. It appeared from their work that neutral fats, from a purely physiological standpoint, are dispensable constituents of a diet, provided a sufficiency of the vitamins frequently found in association with natural fats is contained in the other foodstuffs. The real value of fats as convenient sources of energy is obvious. That the diets used by Drummond and Coward were probably not sufficiently fat-free was shown by Evans and Lepkovsky (1932), who found in ordinary starch enough of an effective substance to prevent the fat deficiency disease in rats. Jaffé (1928) found that mice on a lipid-free diet at first gained, then lost weight and died. There was an increased susceptibility to infections. Whether the bad effects were due to lack of specific fatty acids or to lack of vitamins cannot be said. Schneider, Steenbock and Platz (1940) found that acrodynia in rats could be cured by the essential fatty acids independent of vitamin B_6, or by rice-bran concentrate independent of fatty acids. The concentrate contains, in addition to B_6, a second accessory factor. B_6 alone was ineffective. The Burr and Burr syndrome (see next paragraph) produced by adding dried brewer's yeast to the acrodynia-producing diet could be cured either by the essential fatty acids or rice bran concentrate.

Indications of a fat deficiency disease became manifest through the work of Burr and Burr (1929) and McAmis, Anderson and Mendel (1929). These workers found that the total exclusion of neutral fat from the diet, even if all known vitamins are supplied in adequate amounts, is incompatible with growth and well-being. Burr, Burr and Miller (1932) found that the disease could be prevented and cured by feeding linoleic and linolenic acids. They also reported that arachidonic acid was ineffective in preventing the disease (the opposite result was obtained by Turpeinen, 1937). A variety of other fatty acids have been tried, but linoleic and linolenic acids remain the most satisfactory curative agents.

Hume and associates (1938) found that methyl linolate is much more potent than linolenate, and that hydroxy acids are ineffective. Raisin-seed oil was about as effective as linseed oil, and methyl docosahexenoic was found potent for weight gain but not for the skin lesions. Chaulmoogra oil was ineffective. Nunn and Smedley-MacLean (1938) found that the livers in fat-deficient animals were free of acids containing four or more double bonds, but that arachidonic and docosapentenoic appeared when methyl linolenate was fed. The fact that arachidonic acid was absent from the liver of the fat-deficient animals and that feeding linoleic acid resulted in its restoration indicates that the lack of arachidonic acid was the cause of the deficiency disease and that linoleic acid was a source of arachidonic acid.

That fat-deficient animals have an abnormal fat metabolism is shown by the work of Wesson (1927), in which very high respiratory quotients were obtained in rats on a restricted diet containing no fat, indicating an abnormally great conversion of carbohydrate to fat, apparently in the effort to compensate for the lack of essential fatty materials in the food. This abnormality was not corrected by linoleic acid or by the known vitamins or essential amino acids, but was cured by a substance present in the unsaponifiable fraction of lard (Wesson and Murrell, 1933). It is possible that certain types of obese persons lay on fat because of an increased tendency to form fat from carbohydrate. Wang and Strouse (1925) have reported that obese individuals tend to derive their energy from carbohydrate rather than fat, which may be another expression of the same metabolic preference. The possibility of the lack of some of the vitamins has not been entirely excluded. Birch (1938) reported that vitamin B_6 is a factor in fat deficiency dermatitis, and concluded that the active curative substance was not linoleic acid but some related substance, since the curative action did not run parallel to the linoleic content of the diet.

As perhaps was to be expected, results differing in some respects from those of Burr and Burr as at first reported soon appeared. Sinclair (1932) agreed with them as far as growth was concerned, but not as regards the scaly tail. Graham and Griffith (1931), Hume and Smith (1931), and Funk, Caspe and Caspe (1931) in general supported Burr and Burr's findings. Gregory and Drummond (1932) found that the symptoms of fatty acid deficiency, especially scaly tail, were due to a lack of the vitamin B complex, and stated that since the liver of animals raised on a fat-free diet contained considerable amounts of linoleic or some similar acid the animals still had the power of synthesizing it. Burr and Burr responded that under the feeding conditions employed by these workers, true uncomplicated fat deficiency disease could not result, and that there-

fore the results claimed could not be accepted. That rats, to some extent, can synthesize essential fatty acids is shown by Sinclair (1940). After raising young rats on a deficient diet containing elaidin until they ceased to grow (100 grams weight), the substitution of sucrose for the elaidin gave a rapid weight increase.

Indications at present leave open the possibility that the curative factor in the fat deficiency disease may be one or more unknown substances associated with the effective fatty acids rather than the acids themselves.

Relative value of fatty acids in nutrition. The relative value of various fatty acids was examined by feeding their synthetic glycerides to rats on a limited diet (Ozaki, 1927). Of the saturated acids, lauric and myristic were best utilized; of the unsaturated acids, oleic, linoleic, and linolenic were best, the value decreasing with the number of double bonds. The triple bond acid, stearolic, was toxic. Even-numbered carbon acids were better utilized than odd-numbered ones. Oleic acid was the only one well utilized at the 20 per cent level, lauric and myristic coming next. Palmitic aldehyde was well utilized. The ethyl esters of fatty acids fed at the 10 per cent level appeared to be as well utilized as the glycerides and soaps were quite well used. The specific nature of the fatty acid was found to be important in its utilization.

Oxidation at the double bond. Since unsaturated acids are common constituents of food fats, elimination of desaturation as a stage in fatty acid breakdown does not entirely remove the necessity for considering oxidation at the double bond. It is chemically a point of weakness in the fatty acids, and *in vitro* the unsaturated acids are more readily attacked by oxidizing agents than saturated acids. In the living body, however, there is no evidence that the unsaturated acids are more readily oxidized than the saturated acids, and there is no evidence that the double bond is a point of weakness in oxidation *in vivo*.

If the double bond constituted a point of weakness, oxidation with formation of hydroxy acids and subsequent breaking of the chain would take place in the animal body just as these processes take place in the laboratory with oxidizing agents such as alkaline permanganate. Hydroxy acids, although of relatively frequent occurrence in plants, have been reported in the animal body only in the brain (Grey, 1913), so that they are not of importance as intermediate steps in animals. If the unsaturated acids break at the double bond, each fragment should be oxidizable by itself. The work of Smith (1933) in this laboratory has shown that when oleic acid was broken at the double bond by oxidation, one of the main products was azelaic acid, which was not oxidizable by the animal body. Furthermore, a break at the 9-10 carbon would yield

two odd-carbon fragments which might be expected to yield sugar (Ringer, 1912, 1913); but this is not the case when fats are fed to diabetics (Lusk, 1901, 1908). Furthermore, dicarboxy acids are not formed from the triglycerides of the ordinary food fatty acids, of which the chief one is oleic acid. Oxidations in the animal body are limited by the fact that they must be carried on at a constant and relatively low temperature in a neutral medium and with reagents no stronger than the organic peroxides, so that the analogy with laboratory oxidations cannot be carried too far.

The formation of at least small amounts of dicarboxylic acids in normal metabolism seems probable from the work of Verkade and van der Lee. They found, after feeding triglycerides of various fatty acids of intermediate carbon number, that they obtained small amounts of dicarboxylic acids (1934a). None were obtained from the triglycerides of the higher acids (1934b). The glycerides with nine and ten carbon atoms have the greatest tendency to form dibasic acids (1934a). Evidence was presented (1934c) that the dibasic acids, which represent the first step in oxidation, can undergo the usual β-oxidation with removal of successive pairs of carbon atoms at one or both ends of the molecule. The term bilateral β-oxidation is proposed. They suggest that oxaluria may be the result of this type of oxidation. Oxalic acid occurs in the urine to the extent of 20-40 mg. per day (Müller, 1937).

The behavior of dicarboxylic acids in metabolism has been given some attention. Oxalic acid is resistant to oxidation and is toxic; malonic and succinic acids are readily oxidized, tartaric and glutaric acids less readily; and both these acids produce a severe nephritis (Rose, 1924) as do also to a less extent adipic, pimelic, suberic, azelaic, and mucic (Rose and associates, 1925). Since pimelic (C_7) and azelaic (C_9) acids are not more toxic than adipic (C_6) and suberic (C_8) acids, it is improbable that direct β-oxidation of dicarboxylic acids occurs; otherwise the odd-numbered carbon acids would produce glutaric acid and so cause liver injury. Baer and Blum (1911) reported that adipic, pimelic, and suberic acids had the same effect as glutaric in inhibiting sugar secretion in phlorizinized animals, and probably, as Rose has pointed out, because they are also nephropathic. Rose believed that the toxic action on the kidney is due to slow oxidation. Ringer, Frankel and Jonas (1913) found that malic and succinic acids yielded large amounts of glucose in phlorizinized animals, whereas glutaric acid yielded neither sugar nor acetone bodies. The available evidence thus goes to show that three- and four-carbon dicarboxy acids may be used by the animal body but the higher acids only with difficulty, if at all.

The short-chain fatty acids, when fed to normal animals in moderate

amounts, disappear without trace. Rittenberg, Schoenheimer and Evans (1937) fed sodium butyrate and caproate (labeled with deuterium) to mice and found that they were neither stored nor changed to higher acids, and were therefore burned. When fed to animals rendered experimentally diabetic by phlorizin, the odd-carbon fatty acids yield glucose in proportion to the amount of propionic acid which they would yield by oxidation (Ringer, 1912; 1913), and the even-numbered carbon acids under the same conditions yield acetoacetic and β-hydroxybutyric acids and acetone. Perfusion of the surviving liver with the even-numbered carbon acids caproic, caprylic, and capric yielded acetoacetic acid, but the odd-carbon acids did not (Embden and Marx, 1908).

The action of liver slices on the dibasic acids has been investigated by Mazza (1936), who found that succinic, adipic, suberic, azelaic, sebacic, and hexadecanedicarboxylic acids were oxidized. Glutaric acid was not attacked by liver and very little by kidney. Low respiratory quotients indicated that the initial action was a dehydrogenation. Even-numbered carbon atom acids were more easily oxidized than odd ones. Acetoacetic acid was not produced. It was thought that the oxidation followed the β-oxidation scheme of Knoop-Wieland.

Flaschenträger and Bernhard (1936) reported that the dicarboxylic acids were hard to burn, about 60 per cent of sebacic acid appearing in the urine. Blocking one carboxyl, as in sebacic acid, reduced the loss to 10 per cent. Of the sebacic half ester, 91.8 per cent was burned, 5.2 per cent of the remainder appearing as unchanged ester in the urine, 2.1 per cent as sebacic acid, and 0.26 per cent as suberic acid. These workers conclude that the acids were more easily attacked (by β-oxidation) from one end at a time, and that simultaneous oxidation at both ends of the fatty acid chain did not take place to any important extent.

β-oxidation

The hypothesis regarding fatty acid oxidation most widely accepted at the present time is that of β-oxidation, which involves an oxidation at the β carbon atom followed by the loss of two carbon atoms. This process is continuous until the whole chain is oxidized. The evidence regarding this method of oxidation has been reviewed many times so that only an outline need be given here.

The hypothesis is based largely on results obtained by Knoop (1905) with phenyl fatty acids which showed that the fatty acid side chains were oxidized in this manner. That this method of oxidation is the normal one for the fatty acids is supported by much other evidence. Thus Embden and associates (1906; 1908), by perfusion of surviving livers with blood containing even-numbered carbon chains of 6, 8, and

10 carbon atoms, obtained acetoacetic acid. Odd-numbered acids did not yield this acid. In various conditions, which may be grouped under the general head of lack of available carbohydrate, such as starvation, diabetes, and persistent vomiting, the so-called acetone bodies (aceto-acetic and β-hydroxy acids and acetone) appear in the urine. These substances are known to originate mainly in the fats and would be obtained by the process of β-oxidation from the fatty acids. The objection of chemists that α- and not β-oxidation is the common method of oxidation of the fatty acids was answered by the experiments of Dakin (1908a), who showed that when neutralized butyric acid was oxidized *in vitro* under conditions approximating those in the living body, *i.e.*, with hydrogen peroxide at body temperature, acetoacetic acid and acetone were among the products obtained. When the reaction was carried out at higher temperatures the acetoacetic was converted into acetone by loss of carbon dioxide. He extended the reaction to higher fatty acids (1908b) and found that every normal fatty acid, when neutralized and warmed with hydrogen peroxide, gave the corresponding aldehyde with one less carbon atom. These experiments clearly demonstrated the possibility of β-oxidation *in vitro*.

Clutterbuck and Raper (1925) have made a more detailed examination of the behavior of the fatty acids toward hydrogen peroxide and have found that not only β-oxidation but also ν- and δ-oxidation take place. Their results may be summarized as follows:

(1) The ammonium salts of normal saturated fatty acids when oxidized with hydrogen peroxide yield products which show that oxidation occurs at the more remote carbon atoms as well as the β carbon atoms (and possibly also the α).

(2) The possibility of the occurrence of oxidation in the body at other positions (ν and δ) than the β position is discussed and it is pointed out that if further investigation proves that it does occur, some light may be thrown on the processes by which fat may be utilized to supply energy for the contraction process of muscle.

(3) The first step in the oxidation of normal saturated fatty acids appears to be the formation of a series of keto-acids and not hydroxy acids. This would support the view that the hydroxy acids obtained in experiments *in vivo* result from reduction of keto-acids rather than from direct oxidation of saturated acids.

Kay and Raper (1922) studied the behavior of the branched-chain fatty acids by the use of phenyl-substituted branched-chain fatty acids, feeding them in the same way as Knoop did. Their results were as follows:

Hydratropic acid or α-phenylpropionic acid $\left(\begin{array}{c} CH_3 \\ | \\ C_6H_5.CH.COOH \end{array}\right)$ administered to dogs produced slight toxic effects in doses of 0.25 gram per kilo and was oxidized to the extent of about two-thirds. The remaining one-third was excreted in the urine, partly combined with glucuronic acid. Inactive tropic acid $\left(\begin{array}{c} CH_2OH \\ | \\ C_6H_5.CH.COOH \end{array}\right)$ was very resistant to oxidation, for more than 90 per cent of the acid was recovered from the urine. Inactive atrolactic acid $\left(\begin{array}{c} CH_3 \\ | \\ C_6H_5.C(OH).COOH \end{array}\right)$ was also very resistant to oxidation, over 80 per cent being excreted unchanged. Atropic acid $\left(\begin{array}{c} CH_2 \\ \| \\ C_6H_5C.COOH \end{array}\right)$ was the most toxic of the four acids, but in doses of 0.13 gram per kilo it was tolerated and was completely oxidized. No intermediate products were detected in the urine. Dakin's claim that nearly 70 per cent of phenylacetaldehyde is oxidized in the body was confirmed. Kay and Raper are of the opinion that hydratropic acid is converted directly into atropic acid on oxidation (although this was not directly proved) and that atropic acid then undergoes complete oxidation. "Phenylacetaldehyde largely undergoes complete oxidation in the body and may therefore be an intermediate product in the oxidation of hydratropic and tropic acids." In later work (Kay and Raper, 1924) they found that α-methyl cinnamic yields benzoic acid (33 per cent). Therefore there was rupture of the aromatic nucleus; α-methyl-ν-phenylbutyric yielded phenylacetic acid (50 per cent).

West and Benedict (1925) fed hydroxystearic acid as the ester and found that it was absorbed and partly oxidized since, although ketosis was reduced, the organic acid excretion was little changed. Raper and Wayne (1928) found that normal phenylpropionic, phenylbutyric, phenylvaleric, and phenylcaproic acids are β-oxidized regularly. Phenylnonoic and phenlydecoic acids are less oxidized, which suggests some other method than β-oxidation. Unsaturated acid oxidation is the same as saturated.

According to hypothesis, β-oxidation would result in the successive removal of two carbons until the long chain had been completely destroyed. It is notable that it has not been possible to recover any of these two-carbon fragments, so that their nature is unknown. It would be expected also that unoxidized fragments of different lengths might be recovered under some circumstances. Actually the only fatty acid fragments which are recoverable are the four-carbon fragments, acetoacetic and β-hydroxybutyric acids.

Multiple oxidation and fragmentation

Evidence has been accumulating that successive β-oxidation is not the only mechanism for fatty acid breakdown in the animal body. On feeding various fatty acids and their ethyl esters to rats, Deuel and associates (1935; 1936) found that butyric and caproic acids yielded the same quantity of acetone bodies in the urine as acetoacetic acid, while caprylic acid (C_8) yielded twice as much, indicating a break into two four-carbon fragments with both β- and δ-oxidation. Higher acids gave approximately all possible four-carbon fragments. For example, palmitic, oleic, and stearic acids, fed as ethyl esters, yielded at least three of these four-carbon fragments. Deuel found, in agreement with the work of Ringer and later investigators, that the odd-chain fatty acids did not yield ketone bodies and were apparently oxidized by β-oxidation the same as the even-numbered carbon acids. Jowett and Quastel (1935), using liver slices, found that even-numbered carbon fatty acids produced much ketone body acid but that the odd-numbered ones produced little. The higher liver respiration in the latter cases indicated a more complete combustion than the even-numbered acids. To explain the facts found, Quastel offered a theory of multiple alternate oxidation for the long-chain fatty acids, according to which oxidation may take place simultaneously at the β carbon and at every alternate carbon atom in the chain, with consequent complete destruction of the chain and formation of two- or four-carbon fragments.

By liver perfusion experiments, Blixenkrone-Møller (1938a) found that the respiratory quotient of the livers of normal cats was 0.57 and of depancreatized cats 0.37, the low respiratory quotient being due to the formation of ketone bodies and to a preliminary desaturation of the fatty acids. The amount of oxygen used indicated a breaking of the fatty acids into four-carbon fragments. The ketone body formation varied inversely with the glycogen content. Stadie, Zapp and Lukens (1940) corroborated the work of Blixenkrone-Møller as to formation of ketone bodies in the liver and their destruction in the muscle. Diabetic animals (cats) behaved the same as normals; insulin was not necessary and diabetic muscles could form ketones. The molecular ratio of oxygen consumed to ketone produced was close to 1.25, which indicated the formation of four ketone acids per fatty acid. No oxygen was available for the formation of carbohydrate from fat. Insulin with fructose, fumarate, and *d*-lactate inhibited the formation of ketones by the diabetic liver. In Houssay cats, while fasting, the ketone body formation was essentially normal, from which the conclusion was drawn that insulin controls ketone formation indirectly by acting antagonistically to the ketone pituitary hormone. The low respiratory quotient of cat liver slices (0.32 ± 0.04) agrees with

the conception that most of the oxidations in the liver do not produce carbon dioxide.

The results of these workers obtained from a variety of types of experiment indicate that the hypothesis of β-oxidation with the formation of successive two-carbon fragments may have to be modified to include a process of fragmentation at several points in the chain.

Summary

The mechanisms of oxidation of the fatty acids as proposed at the present time may be summed up in the following two alternative processes:

(a) The long chains are shortened two carbon atoms at a time by successive β-oxidation (probably with the aid of organic peroxides), at least to the four-carbon stage. Here a different type of oxidation involving the simultaneous oxidation of glucose appears to be required in most animals; the resulting two-carbon fragments are oxidized to carbon dioxide and water.

(b) The long chains are completely fragmented into two- and four-carbon residues which are then further oxidized. The liver is apparently the organ in which the fragmentation takes place, and the muscles and other tissues the place where the final combustion takes place. (b) has the greater weight of evidence in its favor.

While the unsaturated acids are more easily oxidized in the laboratory than the saturated acids, there is no certain information that such is the case in the living organism. In some cases, especially with the fatty acids of intermediate chain length, there may be simultaneous oxidation at both ends of the chain. Phosphorization with formation of phospholipids should be kept in mind as a possible early stage in metabolism of fats.

Ketogenesis and Antiketogenesis

According to the hypothesis of β-oxidation of the fatty acids, the long chains are shortened two carbons at a time, yielding each time a two-carbon fragment of unknown nature, which is disposed of by the organism without known difficulty, and an even-numbered carbon fragment of decreasing length, until finally a four-carbon β-oxidized fragment is obtained. This fragment is apparently difficult to dispose of, and under certain circumstances involves the organism in serious trouble. The four-carbon fragment may be either acetoacetic acid ($CH_3.CO.CH_2.COOH$) or β-hydroxybutyric acid ($CH_3.CHOH.CH_2.$-$COOH$), of which the former is regarded as the mother substance. A third compound, acetone ($CH_3.CO.CH_3$), may be formed from the acetoacetic acid by loss of carbon dioxide. These three compounds are known

as the acetone or ketone bodies. According to the more recent conceptions of fatty acid oxidation, these ketone bodies may be formed in larger amounts than by successive β-oxidation. Thus, in Quastel's multiple alternate oxidation as well as in Deuel's system, a number of four-carbon fragments may be formed simultaneously. The difference between the original β-oxidation hypothesis and these later ones is that in the former only one four-carbon fragment is formed, whereas in the latter there may be several. The ketone body acids, especially acetoacetic acid, are sources of difficulty to the organism because, first, they are readily soluble and diffusible and therefore escape from the tissues into the blood and eventually into the urine, and secondly, because they are relatively strong acids, especially acetoacetic acid, and hence require neutralization while in the organism. When they escape into the urine, being strong acids, they carry bases into the urine and, if present in large amounts, may reduce to a dangerous degree the alkali needed to transport carbonic acid. Animals vary a great deal in their ability to dispose of these acid fragments. Dogs, for example, have a nearly perfect mechanism for disposing of them, while human beings have a limited ability. Since the acids are formed mainly from fat, the more fat consumed, the more of these substances is formed. When the production reaches a certain level, they appear in the urine. Normal human beings on a high-fat diet or in fasting excrete ketone body acids. Diabetics, because of their inability to burn carbohydrate and their limited ability to burn protein, have to depend largely on fat for their energy needs and the formation of these acids is great and often dangerous. On the other hand, if the diabetic can be made to burn some carbohydrate, the production of these acetone bodies is reduced or stopped. Out of these facts has grown an enormous literature on ketone acid formation and its prevention (ketogenesis and antiketogenesis).

Formation of ketone bodies (ketogenesis)

In the absence of sufficient available carbohydrate, some animals, including man, are unable to oxidize unlimited amounts of the fatty acids past the four-carbon stage, and the unburned products, acetoacetic acid, β-hydroxybutyric acid, and acetone (ketone or acetone bodies) appear in the excretions. Recent investigations (Blixenkrone-Møller, 1938c) indicate a much larger production and utilization of these substances in the normal individual than was previously believed. The ketone bodies are apparently formed only in the liver and are consumed mainly in the muscle and kidney. Comparison of the liver output and urine output showed that there was a large production of ketone bodies, only a small fraction of which appeared in the urine, the remainder being burned;

and it seemed probable that this was an important method by which the fats furnished energy to the muscles. The ketone body production varied inversely with the glycogen content of the livers, a fact earlier reported by Raper and Smith (1926). Toenniessen and Brinkmann (1938) also found that the liver supplies acetic and ketone body acids to the muscles, which then burn them. Terashima (1937) showed that the kidneys could form ketone bodies from both fat and amino acids and could burn these substances as well, indicating that the kidneys are metabolic as well as excretory in this respect. In normal human blood, Marriott (1914) found about 4 mg. of β-hydroxybutyric acid and 1.5 mg. of acetone plus acetoacetic acid per 100 cc. of blood. In diabetic coma, the figures were 45 and 28 mg. per 100 cc., respectively.

While fat and fatty acids are regarded as the main source of acetone bodies (ketogenic substances), certain amino acids of the protein molecule may furnish some, as the following study by Embden, Salomon and Schmidt (1906) shows. The study consisted in perfusion experiments on the surviving liver in which the substance being tested was added to the perfusing fluid and the acetone formed measured in milligrams per liter. Two grams of glycocoll yielded 24 mg. acetone per liter; 5 grams alanine 15 mg.; 2 grams ammonium glutamate 15 to 23 mg.; 5 grams asparagine 20 mg.; 5 grams leucine 55 mg.; 2 grams *n*-α-amino-caproic acid 20 to 23 mg.; 2 grams α-amino-isovaleric 11 to 27 mg.; and 2 grams of isobutyl acetic acid 15 to 24 mg. These amounts are near the limit of error of the experiment and therefore doubtful. Two grams of isovaleric acid yielded 73 mg. of acetone. The three homologous acids—isobutyric, isobutylacetic, and isovaleric—behaved as though their chains were attacked like Knoop's compounds. Leucine, after losing its carbon dioxide, behaves like isovaleric, and amino-isovaleric like isobutyric. Oxybutyric acid is a strong acetone former (130-160 mg.) and normal butyric is very active. Two and one-half grams tyrosine gave 68 to 104 mg.; 2 grams phenylalanine 37 to 88 mg.; 2 grams α-phenyllactic acid 45-52 mg.; and 2 grams homogentisic 39 to 68 mg. β-Phenyllactic, phenylacetic, phenylpropionic, and cinnamic acids were not acetone formers in the liver. In general, only those aromatic compounds of which the benzene ring can be destroyed in the animal body yield acetone bodies.

As antiketogenic substances may be listed glucose or any substance which yields glucose in metabolism, such as part of the protein molecule, the glycerol of fats, lactic acid, etc.

Since it is the even-numbered fatty acids which produce the acetone bodies, considerable interest was aroused by the announcement of Kahn (1923) that an artificial glyceride has been prepared from the odd-carbon margaric acid (C_{17}). This substance, called "intarvin," was claimed to

be non-ketogenic, the final result of β-oxidation being propionic acid, which, according to Ringer, formed glucose. Keefer, Perlzweig and McCann (1924), reporting on the use of this compound in human diabetes, found that intarvin was apparently less ketogenic than ordinary fat, but the evidence was not entirely conclusive. From a practical standpoint, the disagreeable taste of intarvin fat was sufficient to detract very much from its value. Results divergent from those of Kahn were obtained by Lundin (1923), by Modern (1923), and by Lyon, Robson and White (1925). Carbohydrate and insulin requirements were the same as for the even-carbon acids, although the acid products were lactic and pyruvic acids instead of the ketone body acids. It is interesting from a biochemical point of view that Kahn, confirmed by most other workers, found that this artificial and abnormal (in that it consists of odd-numbered carbon fatty acids) fat was absorbed by the human organism to the extent of 90 per cent, that it allayed hunger and stopped the loss of weight and so was apparently utilized. It should be noted that β-oxidation was the rule with these acids also.

Hormonal regulation of ketone body formation. Considerable evidence is available that the ketone body formation by the liver is under the control of a pituitary hormone. Burn and Ling (1933) found that injection of extract of anterior pituitary into female rats on a fat diet caused a great rise in acetone body output. They concluded that ketonuria depended on hormones in the blood rather than on any relation between fat and carbohydrate in the diet. This finding has been repeatedly confirmed for the rat; it has also been found in the dog by Rietti (1934) and the guinea pig by Best and Campbell (1938), who also found that the hormone increased the liver fat. It is uncertain whether the anterior pituitary hormone is specific for fat or acts indirectly by suppressing carbohydrate and protein metabolism as indicated by the work of Russell (1938). Shipley and Long (1938) do not believe that there is a special ketogenic enzyme. They think that the well-known effects of anterior pituitary extract on carbohydrate and protein metabolism are sufficient to account for the extra fat metabolism which produces these effects. Harrison and Long (quoted from Shipley and Long) observed a 30-40 per cent reduction in urine nitrogen in the fasting rabbit on treatment with anterior pituitary extracts.

Thyroid or dinitrophenol administration increased the utilization and increased the output of ketone bodies (Mirsky and Broh-Kahn, 1937), indicating a balanced control of the ketone bodies by the hormones of the pituitary and thyroid. Remington (1938) reported that adding fat up to 30 per cent of the caloric intake did not aggravate the hyperplasia of the thyroid due to iodine deficiency.

The effect of adrenalectomy on ketosis was investigated by MacKay and Wick (1939), who found that the operation raised the kidney threshold for ketones, and that there was only a slight lowering of ketone production in fasting after adrenalectomy. Nelson, Grayman and Mirsky (1940) found that adrenalectomy reduced the rate of ketone body utilization and probably of formation also, but considered that both might be due to a general decrease in metabolic activity.

The pituitary hormone regulating fatty acid mobilization to the liver and its transformations there may be the same one which by its absence produces the obesity characteristic of Fröhlich's syndrome, probably because of the inability to move the fat from the depots into the blood and possibly from the blood to the liver.

Acid intoxication

The condition of acid intoxication, due to depletion of fixed alkali, is almost always associated in man with incomplete combustion of fatty acids. The symptoms are, however, essentially the same as those produced by incombustible acids of any kind. Excess of inorganic acids, such as hydrochloric or phosphoric which cannot be destroyed, and must be neutralized and excreted, produces in man the following symptoms: stupor, coma accompanied by excessively active respiration (air hunger) with normal oxygen content and low carbon dioxide values in the blood. The urine contains increased quantities of calcium, magnesium, potassium, sodium, and ammonium. In dogs, ammonia alone is much increased, and this animal is relatively resistant to acid poisoning. Acidosis is therefore an impoverishment of the blood and tissues in fixed bases, reducing their capacity to combine with and eliminate carbon dioxide and other acids normally formed in metabolism and resulting in asphyxiation due to retention of the body's own products of combustion. Practically, acidosis in man results either from defective oxidation of organic acids formed in metabolism or defective elimination of various acids, originating in the food or in metabolic processes due to impaired kidney function. The most important source of organic acids is incompletely oxidized fatty acids; in man, acid poisoning by these substances is not an infrequent occurrence, although confined largely to one disease, diabetes mellitus. In this disease the final stage is often coma in which the symptoms are strikingly similar to those noted above for acid poisoning: asphyxia due, not to diminished ability of the blood to carry oxygen, but to a diminished ability to carry carbon dioxide caused by depletion of fixed alkali.

Removal of ketone bodies (antiketogenesis)

Simultaneous oxidation with glucose. The necessary amount of

actual or potential carbohydrate in the diet of human beings in order to avoid "acidosis" or "ketogenesis" has been the subject of many investigations, of which the most extensive have been those of Woodyatt (1921) and Shaffer (1923). Without going into the details of this work, which has been reviewed by Shaffer (1923), the results may be summed up as follows:

One molecule of glucose or its equivalent in other substances which yield sugar in metabolism (antiketogenic substances) is theoretically able to bring about the complete combustion of two molecules of fatty acid or other substances yielding ketone compounds (ketogenic substances); but probably because of uneven distribution and uneven metabolism in different parts of the body, it is necessary, in order to be certain of avoiding the production of the acetone derivatives, to allow in the diet one molecule of antiketogenic substance, such as glucose, to one molecule of ketogenic substance, such as fatty acid. These results have already been applied satisfactorily in clinical practice (Wilder, 1922; Hannon and McCann, 1922; Hubbard and Nicholson, 1922), which is proof of their essential soundness.

Shaffer has contributed much toward the elucidation of the manner in which the carbohydrates assist in the combustion of these fragments of the fatty acids. He found that the combustion (Shaffer, 1921; Shaffer and Friedemann, 1924) of diacetic acid in the presence of glucose is probably preceded by a condensation of the Knövenagel type between some derivative of glucose and the diacetic acid, the condensation product being much more easily oxidized than the diacetic acid alone. The details of this work by Shaffer and his co-workers are reviewed by West (1925; 1927). It was found possible to produce addition products of diacetic acid with glucose and other aldehydes which were much more powerful reducing agents (*i.e.*, more readily oxidizable) than glucose itself.

Hubbard and Nicholson (1922) found in diabetics that the acetone excretion varied inversely with the numerical values of the ketogenic-antiketogenic ratio. A study of the numerical values of the ratio calculated for diets, which corresponded to a slightly increased excretion of acetone, showed that they were approximately the same as those values found for normal subjects receiving diets low in carbohydrate but containing sufficient calories to supply the needs of the subject. It was shown that fed fat sometimes increases the amount of acetone excreted, even when the increase replaces a part of the fat which the body was probably withdrawing from its own reserve supplies of this material. Dye and Chidsey (1939) reported that the rate of ketone body utilization by the tissues was not influenced by the blood sugar level, total carbo-

hydrate utilization, evisceration, or pancreatectomy but by their own concentrations in blood and tissues.

A relatively simple explanation of the phenomena of acidosis in diabetes has been suggested by MacCallum (1930). It seemed probable to him that the occurrence and excretion of the acetone bodies was only indirectly concerned with the combustion of carbohydrates and was rather the result of the necessarily excessive combustion of fat. Since each fatty acid chain results in at least one molecule of either β-hydroxybutyric or acetoacetic acid, the amount of these substances formed when mostly fat is being burned is relatively large—two or more times as much as is formed normally. These acids, especially acetoacetic acid, are quite strong, and must be almost completely neutralized. They are also readily diffusible. Unless quickly burned, they would therefore escape into the blood and urine; and, if the ability to burn them is limited, as it apparently is, their appearance in the urine and their effects on the alkali reserve would be the same as that produced by the equivalent amounts of any strong acid. It would be analogous to the formation of large amounts of lactic acid in severe muscular exercise. If carbohydrate can be burned, it eases the strain on the fat-burning mechanism and so cuts down on the excessive acid production. On this basis, adaptation to a high-fat diet is readily explained. The ketogenic:antiketogenic ratio may simply represent the limit of the rate at which the human organism can burn fat. By adaptation, this rate can be increased, and in animals such as the dog the adaptation has long been established.

However, it may be pointed out that the glucose required to prevent ketonuria is not equivalent in calorie value to the fatty acid, so that it is probably something more than caloric replacement that accounts for the effect of glucose on the acetone bodies. Evidence on this point has been offered by Deuel, Hallman and Murray (1938), who compared ethyl alcohol with glucose in its antiketogenic effects on high-fat or sodium butyrate diets. If it were only a matter of caloric substitution, a substance like alcohol which burns to furnish energy in metabolism should be as effective as glucose in preventing ketosis. It was found that, with the same amount of fat oxidized, glucose prevented ketosis but alcohol did not; and the conclusion was that glucose had a specific function in ketolysis aside from its caloric influence. It seems possible that the specific action of glucose may be the result of the much discussed glycogen-fat antagonism in the liver. As long as there is a minimum of glycogen present in the liver, there will be little ketone body formation, and any procedure which would affect this glycogen store would affect the ketone body production. Thus, feeding even a small amount of glucose,

the use of insulin, etc., might produce an effect on the ketonuria far greater than would be expected from the amount of glucose involved.

Effect of insulin. In experiments on normal animals, Hawley and Murlin (1925) found no rise in respiratory quotient after administration of insulin, but often a fall lasting up to one hour after dosage, accompanied by an increase of metabolism. There was then a rapid rise to 0.98 in the second hour, and later a fall to the pre-insulin values. The increase in metabolism observed after insulin during the first hour is therefore due mainly to combustion of fat, and in the second hour to carbohydrate. In agreement with the above findings, Collip (1923) makes the statement that while we have in insulin a substance which will correct all the acidotic signs, both in experimentally diabetic animals and in patients suffering from diabetes (if the dosage is adequate), it will nevertheless produce many of the cardinal symptoms of acidosis when administered to the normal animal in amounts sufficient to produce a hypoglycemia of a marked degree.

In perfusion experiments, insulin did not inhibit the production of acetone bodies in blood perfused through surviving cat livers, the extent of which was found to depend on the fat content of the liver and to vary inversely with the glycogen content. Sodium butyrate added to the blood greatly increased the acetone body production (up to 80 per cent of the theoretical) and was not affected by insulin (Raper and Smith, 1926).

Illuminating evidence on the production of acetone bodies is supplied by Cori and Cori (1927). They found a seasonal occurrence of ketonuria in rats with excretion of over three times as much ketone bodies in summer as in winter. This was correlated with a lowering of glucose tolerance by about 36 per cent in the summer. Recovery experiments showed that the lower tolerance was due to a lessened ability to oxidize glucose which could be increased with insulin. There appeared to be a reduced functional activity of the pancreas in the summer.

Adaptation. The dog, and possibly carnivorous animals in general, are immune to acid intoxication, a result probably of long adaptation to the presence of considerable fat in their diet. Instances of similar adaptation of human beings and other animals to a high-fat diet are available in the literature. Thus Wigglesworth (1924), in experiments on rats on an exclusive fat diet, found marked ketosis which reached a maximum on the third day, then subsided, and adaptation was complete on the fifth day. Sodium bicarbonate (6 per cent) prevented the adaptation, causing an increased output of lactic and hydroxybutyric acid, indicating either that alkali interfered with the normal oxidation of fat, or that the acids were used as a protective mechanism against the excess of alkali. Folin and

Denis (1915) found that obese individuals, when fasted, excreted less acetone bodies on the second and succeeding fasts than on the first one. Hawley, Johnson and Murlin (1933) found that normal human beings vary a good deal in their tolerance for high-fat diets, and many of them show increased tolerance with time.

A possible sex variation in ability to burn these compounds was indicated by Deuel, Hallman and Murray (1937). They found that female rats produced more ketone bodies than males; but just the opposite was found for cats by Chamberlin, Furgason and Hall (1937). Male cats on beef heart secreted more ketone bodies than females. Castration abolished the difference, which was restored by testosterone. A mixed diet also abolished the difference.

In view of the danger of acid poisoning from incomplete combustion of fat, it is all the more interesting that a diet very high in fat (220 grams per day or over) has been found by some clinicians to give very satisfactory results in diabetes. Newburgh and Marsh (1921) found that the high-fat diet not only stopped the glycosuria but reduced the level of blood sugar to normal. Maignon (1922) found that the administration of abundant fat in diabetic acetonuria was free from danger, and even diminished the acetone body output if alkali was supplied to combat the urinary hyperacidity. Hubbard (1923) found that on diets low in antiketogenic material ingested fat and body fat gave the same amount of acetone bodies. Petren (1924) fed very high-fat diets with good results. As MacCallum (1930) suggests, the *ketogenic:antiketogenic* ratio may simply represent the limit of the rate at which the human organism can burn fat, and, by adaptation, this rate might be increased.

On the other hand, Allen (1923) found that the high-fat diet was dangerous to dogs made diabetic by partial pancreatectomy. They did well at first, and gained in weight, even up to nine months, on the high-fat diet. Then glycosuria appeared, they lost weight rapidly, and finally died from extreme weakness. Acidosis, which is difficult to produce in dogs, becomes possible after sufficient of the pancreas has been removed to render them diabetic. With active diabetes, a high-fat diet produces acidosis and they may even die in coma. There is a possible relation between these results and the work of Best and his collaborators, who found that depancreatized dogs could not be kept alive on insulin alone. They did well for a while, then began to fail, and often died quite suddenly. The outstanding abnormality found at autopsy was a fatty liver. Further experiments showed that this condition could be prevented by feeding various substances: raw pancreas, lecithin, and later choline. It is significant that the present tendency in diabetic dieting is away from the high-fat diets.

Neutralization and excretion. The tissues and especially the blood are normally kept at a reaction very near the neutral point (pH 7.3 to 7.4) and the extreme variations allowable with life are only a few tenths either way. To keep the pH of blood and tissues within the narrow limits of reaction noted and to keep a sufficient "alkali reserve" to take care of respiration and acid production requires the use of various devices in the animal economy. The more important ones are:

(1) Excretion of an acid urine; the limit in humans appears to be a pH of about 5 (Henderson and Palmer, 1913). This is accomplished largely by the use of acid phosphate.

(2) Neutralization of the acids by ammonia, making use for the purpose of nitrogen which would otherwise be excreted as urea (Fiske and Sokhey, 1925).

(3) Use of alkali in the food or of foods yielding an alkaline ash.

(4) As a last resort, the use of the fixed alkali of the blood and tissues down to a point where respiration can no longer be carried on.

Animals vary a great deal in their ability to protect their fixed alkali. Herbivorous animals have little power of resistance to acids, but carnivorous animals, such as the dog, are very resistant by reason largely of their ability to use ammonia for neutralization. Man comes in between these two extremes; and when carbohydrate is not present in the food or cannot be made available from it, as in diabetes, he is in danger due to depletion of the alkali necessary for respiration.

The excretion of the ketone body acids in man often reaches 15 to 20 grams per day, and as much as 150 to 200 grams have been claimed. According to Magnus-Levy (1908) the largest amount of β-hydroxybutyric acid which can be formed from 100 grams of fat is 36 grams, *i.e.*, one molecule of the acid from each molecule of fatty acid. β-hydroxybutyric acid is normally 60 to 80 per cent of the total (Kennaway, 1914), although acetoacetic acid is believed to be the mother substance. The change from the relatively strong acetoacetic to the much weaker β-hydroxybutyric may be a protective mechanism, since the weaker acid would require less base for excretion.

Since the formation of β-hydroxybutyric and acetoacetic acids and the necessity for their neutralization is one of the main problems in the treatment of diabetes, the possibility of feeding alkali to help the organism with this neutralization was suggested and tried early. The unexpected result was that it often increased the formation of these acids. Why this happens depends apparently on two factors:

(1) The inhibition of an adaptive mechanism which, in some cases at least, would have taken care of the situation by combustion of the acids as Wigglesworth (1924) found with rats; and

(2) The presence of excess alkali which calls for acid to preserve the reaction of the body fluids.

Sodium bicarbonate was the alkali most frequently used. Other salts and ions were tried by Takao (1926); calcium decreased it, sodium was without effect, while ammonium, potassium and magnesium increased it.

Effect of High-fat Diet

Cathcart (1922), working with a human subject, found that the total nitrogen, urea, and ammonia rose on a fat diet (323 grams olive oil daily emulsified with a little potassium carbonate), while uric acid fell. Creatinin was little affected, but there was some excretion of creatin. The addition of carbohydrate caused a fall of the nitrogen excretion and disappearance of creatin. The subject found it impossible to carry on for more than three days on this very high fat diet because of nausea.

Gräfe and Weissmann (1924) found that there was a loss in the body weight and a rise in total metabolism in dogs on a diet high in fat, as had also been reported with a high carbohydrate diet. However, the results in regard to the dynamic action and the water balance are not so striking on the high-fat diet as those on the high carbohydrate diet.

Murlin and Lusk (1915), in a study of the course of metabolism after fat ingestion, found a rise in fat metabolism comparable with the rise of blood fat. Ingestion of fat increased the heat production but did not change the quantity of heat produced from protein and glycogen. Glucose with fat was additive.

Levine and Smith (1927) found that growth at a normal rate, from 30 to 180 grams of body weight, could be induced in rats on a diet devoid of carbohydrate and containing 86 per cent of the calories as fat. The livers of these animals did not contain fat droplets. Growth efficiency was quite as good when measured by energy cost. Ingested fat was utilized to the extent of 98 to 99 per cent. Holt and Fales (1923) found that a marked reduction of fat in children's diets resulted in excessive fermentation and an increased output of calcium in the feces. A diet with fat enough in it to produce ketosis caused increased blood uric acid, according to Harding and co-workers (1925).

Metabolism of Cholesterol

Very little is known regarding the behavior of lipids other than fat in metabolism. As far as is known, phospholipid behaves like the other closely related triglycerides, the fats. Cholesterol appears to be necessary for life and if not supplied in the food in adequate amounts may be synthesized. Any excess is generally excreted. Recent work by Page and Menschick (1932) indicates that considerable amounts of cholesterol

may be destroyed, and Beumer (1925) found that the total cholesterol of puppies (fecal included) did not change during a fifteen- to twenty-day fast, and that therefore there was no combustion of cholesterol in these animals.

Excess of cholesterol in the food is excreted partly unchanged in the bile, partly by the intestine. McMaster (1924) found that the amount of cholesterol in the bile was greatly increased by feeding cholesterol, while in fasting the output was greatly reduced. D'Amato (1915) found that cholesterol feeding (in the form of brain and egg) caused a small but constant increase of cholesterol in the bile, but the increases were so small as to indicate that the bile was not the main path of excretion. Gardner and Gainsborough (1930) found that cholesterol is eliminated by the liver and to a considerable extent reabsorbed from the intestine. In man there is ordinarily a negative balance of 0.3 gram per day, which probably represents the daily synthesis. Schoenheimer and Sperry (1934), by using dogs with bile fistulas thus eliminating excretion by the bile, were able to show that sterols were excreted by the intestine, largely as coprosterol. Kaufmann and Mühlbock (1933) report that in pregnancy, in spite of hypercholesterolemia and the requirements of the fetus, there is always more cholesterol excreted than is ingested, indicating a considerable synthesis. Congenital inability to excrete cholesterol is characteristic of the disease essential xanthomatosis (Schoenheimer, 1933).

Sperry and Stoyanoff (1935) found that cholesterol fed to chickens was deposited in the liver as in rats but proportionately less was deposited as esters. Rats fed cholesterol grow and eat less well, utilize their food less well, and do not show more resistance to infection.

Plants form sterols actively during germination and growth (Beumer, 1933).

Fat Metabolism in the Developing Embryo

Hen's egg

Needham (1931) sums up the behavior of the lipids in hen's egg during development as follows: The ether-soluble fatty substance diminishes from 5.4 grams to 2.73 grams during incubation. In the early days (seventh to fourteenth) the fat lost, as determined by chemical analysis, is in excess of that determined by the carbon dioxide output even if all of the carbon dioxide comes from fat, which is not the case. Therefore, some of the fat is transformed into other substances, possibly carbohydrate. All curves of lipid content have a rather sharp inflection at about the fourteenth day, indicating an awakening of fat metabolism toward the end of the incubation period. This corresponds to the observation that there is an important rise in the body fat of the embryo during

the week before hatching. Gage and Gage (1908) found that stained yolk fat was not deposited in the embryo until the seventeenth day of incubation. Cahn (1928) found that the triglycerides in the embryo increased during the incubation and more sharply after the fourteenth day. Eaves (1910) found that the iodine number of the fatty acids of the chick rose during development after the tenth day, while that of the rest of the egg material fell, *i.e.*, there was either a selection or a desaturation of the fatty acids by the embryonic tissue. To test the power of desaturation by the embryonic tissues, an aqueous emulsion of embryo tissues was mixed with yolk and incubated for five days at 37°C. There was a marked increase in the iodine number of the yolk fatty acid in the case of the twelve- and nineteen-day embryos and yolk, but none in the six-day ones. Apparently the early embryo cannot desaturate the fatty acids.

The change in the phospholipid content of the hen's egg during development has been quite fully worked out, first, by Plimmer and Scott (1909), and later by Masai and Fukutomi (1923). They found that the phospholipids (ether-soluble phosphorus) remain practically unchanged until the fourteenth day, when they begin to diminish rapidly, inorganic phosphorus increasing proportionately. Riddle (1916) found a rapid absorption of phospholipid from the yolk and yolk sac of the chick during the last week of incubation. After the twelfth day the phospholipids are utilized more rapidly than the neutral fats, and the neutral fats than the proteins.

Cahn's (1928) studies on the lipid phosphorus during development furnish a good picture of the phospholipid changes in the embryo as distinct from the whole egg. The total amount of phospholipid rose steadily, but the neutral fat did not increase much until about the fourteenth day, when there was a rapid rise (in this respect paralleling the fall of phospholipid in the yolk and the accumulation of fat in the liver of the chick noted above). The changes in cholesterol fell about midway between those of the phospholipid and fat. After hatching, there was a rapid rise in phospholipid. In terms of dry weight, there was an increase to a maximum at about the tenth day and then a decrease. The curve of daily increment of phospholipid reached a maximum at about the fourteenth day, then fell off rapidly. The peak in the increment curve of cholesterol was not reached until about three days later. Kugler (1936) investigated the changes in the phospholipids during development in greater detail. Lecithin and cephalin parallel each other in both yolk (decrease) and embryo (increase), preserving the initial relation of 3 lecithin to 1 cephalin throughout. Ether-soluble lipid phosphorus was always about 18 per cent greater than the sum of

the phosphorus of lecithin plus cephalin, a difference which Kugler ascribed either to losses inherent in the method or to partial hydrolysis of the phospholipids. (This difference which is still higher in other tissues [Le Breton, 1921] constitutes a serious objection to determining phospholipid as ether-soluble phosphorus.) Hevesy, Levi and Rebbe (1938) introduced radioactive phosphorus as phosphate into the egg before incubation and then examined its distribution after 6, 11, 16, and 18 days. They found that the phospholipid in the embryo was highly active while that in the yolk was inactive, indicating that the embryo synthesized its phospholipid anew rather than absorbing the yolk phospholipid without change.

Hanes (1912) made a histochemical study of the fatty changes in the chick liver during incubation and found that at or about the momentous fourteenth day, cholesterol esters begin to accumulate in the liver, which fact he believed could be linked up with the drop in the yolk phospholipid which takes place at this time. The four fatty acid molecules which are set free by the breaking down of two phospholipid molecules recombine to form a molecule of fat, and the extra fatty acid combines with a molecule of cholesterol to form cholesterol ester schematically as follows:

```
        /F.A.                                   /F.A.
Glycerol—F.A.     + Cholesterol = Glycerol—F.A. + Cholesterol F. A. +
        \P-choline                              \F.A.
Glycerol-P-choline residual.
```

Needham (1931) makes one objection to this scheme—why does the cholesterol not combine with the fatty acids while in the egg yolk?—to which the probable answer is that the fatty acids in the yolk are not free but in combination as neutral fat or phospholipid, and the cholesterol ester is not formed until the phospholipid is broken down, about the fourteenth day, liberating its fatty acid.

Mueller (1915) studied the free and bound cholesterol in the whole egg during incubation and found that while the total cholesterol remained constant, the ester cholesterol increased from practically none up to and at the thirteenth day, to about 40 per cent at hatching, thus supporting the findings of Hanes. After hatching, Mueller found a fall in total cholesterol and cholesterol esters, the latter also in agreement with Hanes. Mueller thought that the esterification of the extra fatty acid was of the nature of detoxication, the free acid being possibly harmful. His results were later fully confirmed by Dam (1929).

The possible synthesis of cholesterol during incubation of the egg has been investigated by several workers, but the question is left in doubt. Roffo and Azaretti (1926) and Ellis and Gardner (1909) found no certain synthesis, but Channon (1925), Thannhauser and Schaber (1923) and

Dam (1929) found evidence of synthesis. In terms of the dry weight of the developing embryo, there appeared to be a maximum content of cholesterol at about the eleventh day (Roffo and Azaretti, 1926; Cahn, 1928) and then a falling off, which would link it up with the observed accumulations of cholesterol as ester in the chick's liver found by Hanes. Other work in this field is that of Kusui (1929, 1930), which confirms the esterification of cholesterol in the later stages and indicates that the total cholesterol changes little, if any. He found similar changes in marine turtle eggs. Entenman, Lorenz and Chaikoff (1940) found that the liver of the newly hatched chick contained large amounts of cholesterol, mainly as ester (up to 9.7 per cent total cholesterol which was about one-half the total lipid). The amount diminished rapidly until at the end of the first month it was at the adult level (about 0.4 per cent). Free cholesterol remained quite constant so that the main changes were in the esters. Phospholipid fatty acids remained constant throughout. Between the second and seventh day, there was a rise in neutral fat along with a fall in cholesterol esters. In the blood, the lipids were high (1 per cent) diminishing at fifteen days to 0.452 per cent. The yolk sac, which was a diverticulum of the small intestine, contained at hatching 12 per cent of fatty acid, 1.6 per cent of phospholipid, and 1.3 per cent of total cholesterol. Absorption of this material was nearly complete in five days, the neutral fat disappearing most rapidly.

The coefficients of Mayer and Schaeffer have been examined in connection with embryonic tissues by Needham (1931, p. 1226). The lipocytic coefficient, *total fatty acid/total cholesterol,* when multiplied by the maximum water retained by 1 gram dry weight of tissue, gives a constant value of 60. The value of this coefficient was found to be much higher in embryonic than in adult tissues, thus giving support to Mayer and Schaeffer's generalization. The coefficient, *lipid phosphorus/total cholesterol,* was found by Cahn to be very constant throughout development.

Mammalian embryo and placenta

Kreidl and associates (1910) found that the fat content of the fetal guinea pig's blood was 746 mg. per cent as compared with 314 mg. per cent for the maternal blood. Slemons and Stander (1923) found differences between maternal and fetal blood in humans. In blood plasma their average values for total lipid were: maternal 942 mg. per cent; fetal 737 mg. per cent. Bailey and Murlin's (quoted by Murlin, 1917) figures on humans were: maternal 550 mg. per cent; fetal 270 mg. per cent total lipid.

Stained fat fed by Gage and Gage (1909) to pregnant animals of different species was never found in the fetus. Mendel and Daniels

(1912) and Baumann and Holly (1926) likewise obtained negative results in this type of experiment. Wesson (1926) fed cod liver oil to one lot of pregnant rats and butter to another and found almost no difference in the fetal fat as shown by the bromine absorption numbers. He concluded that the fatty acids do not pass the placenta at all and that the embryo forms its fat by synthesis from other sources, presumably carbohydrate. Since it is pretty well established that fat synthesized from carbohydrate has a relatively high melting point (see Ellis and Zeller, 1930), and since the fat of the fetus is generally high-melting, his conclusion seemed to fit the facts. Other work, however, goes to show that the placenta of some animals is permeable to fat. Thus Bickenbach and Rupp (1931) have demonstrated that rabbit placenta is permeable to the unsaturated acids of linseed oil, and Sinclair (1933) found that the placenta of the rat was permeable to the unsaturated acids of cod liver oil. Thiemich (1905) obtained positive results on the dog. Boyd and Wilson (1935) found that whole blood from the umbilical artery at birth contained 22 per cent less phospholipid and 14 per cent less free cholesterol than whole blood from the umbilical vein. The results were considered to signify that phospholipid and free cholesterol were regularly absorbed by the human fetus from umbilical blood at birth. Neutral fat could be either absorbed or given up while cholesterol esters could be absorbed if over 10 mg. per cent was present.

Regarding human fat at various ages Raudnitz (1888) found that its melting point was higher the younger the body. Thus:

	M P. (°C.)
8.5 month fetus	47 2
2 days after birth	43 8
1 year 11 months old	28 7–30.2
26 years old	27

Jaeckle (1902) gave the following figures for the distribution of the fatty acids in body fat (from Langer):

	At Birth (%)	Adult (%)
Oleic	65.0	86.2
Palmitic	28.0	7.8
Stearic	3.2	1.9

Imrie and Graham (1920) made a study of the liver fat in guinea pig fetuses at different ages and brought out the significant fact that there is an accumulation of fat in the fetal livers toward the end of gestation, and since this store diminishes rapidly in the hours after birth, it is likely that this accumulation is a preparation for the emergency of birth. The iodine value of the liver fatty acids does not change significantly, although it is always higher than the stored fat of these animals and always higher than that of the maternal liver. Hentschel

(1932) found, in the human fetus, that while the absolute total sterol increased up to birth, the relative content did not change. The brain sterol increased with development. In the skin, the content was highest in the early stages, but there was a steady absolute increase with development.

Further discussion of the transfer of material through the placenta is given in Chapter VI, page 351.

The Vitamins in Lipid Metabolism

Very little work has been done on the influence of vitamins on lipid metabolism. Vitamins A, D, and E are known as fat-soluble vitamins, *i.e.*, they occur naturally associated with the lipids, but with the exception of vitamin A have little to do with their metabolism.

Asada (1923, 1924) reported high blood lipids in rats on a vitamin A-deficient diet, which indicated a disturbance in the power of the body cells to take up fat when vitamin A was lacking. Liang and Wacker (1925) found that in the absence of vitamin A the growing rat cannot form fat from carbohydrate nor can it synthesize fatty acids and glycerol to fat. Cholesterol, however, is synthesized in the absence of fat or vitamin A. Emerique (1932) found that fecal lipids were the same on a 2 per cent or a 20 per cent fat diet in vitamin A-deficient rats, and concluded that fat absorption is not affected by lack of vitamin A. Vitamin A deficiency was found to produce a considerable decrease in the phospholipid fatty acid content of liver, lung, kidney, and spleen (Monaghan, 1932).

During attacks of beriberi in pigeons, Lawaczeck (1923) found the cholesterol high in blood and skeletal muscles. Hotta (1923, 1924) found increased cholesterol in all body tissues except kidneys and testes. Cholesterol fed to beriberi pigeons changed the symptoms of the disease markedly. Mottram, Cramer and Drew (1922) found that the presence of B complex had a profound influence on the passage of fat through the intestinal wall, being absorbed as a stream of fine droplets running through the cells when B complex was present; otherwise the absorption was in large drops. Absorption by streams or by drops could be produced at will. Lecoq (1933) found that the assimilation of lipids by the organism required the presence of B complex, and Lecoq and Savore (1933) found that avitaminosis B comes on more rapidly in the presence of digestible fats.

In experimental rickets in dogs, Sharpe (1922) found that the phospholipids of the blood and liver were lower in rachitic than in normal pups and that the proportion of liver phospholipid to blood phospholipid is lower in rachitic animals than in normal. His idea was that the phos-

pholipids were concerned in the phosphorus supply to the bones, which is known to be the case in the developing chick embryo.

In scurvy, the changes in blood and most tissue lipids are generally insignificant (Nagayama and Tagaya, 1929), but there is a marked drop in the unsaponifiable substance in the adrenals. For example, in controls the value was 7.44 per cent, but in the scorbutic animals the values were 2.12 per cent accompanied by great hypertrophy. The decrease in unsaponifiable was due mainly to cholesterol.

Adams (1936) found an increased phospholipid content of the skin of vitamin G-deficient animals.

Bibliography

Adams, P. D., *J. Biol. Chem.*, 116: 641 (1936). p. 343
Allen, F. M., *J. Metabolic Research*, 3: 797 (1923). p 334
Allin, K. D., *see* Harding, V. J.
Anderson, I. A and Macleod, J. J. R., *Biochem. J.*, 24· 1408 (1930). p. 305
Anderson, W. E , *see* McAmis, A J.
Anderson, W. E. and Mendel, L. B., *J. Biol. Chem.*, 76: 729 (1928). p. 287
Arinstein, B , *see* Neuberg, C
Artom, C., *Arch. fisiol* , 32: 57 (1933). p 296, 300
Artom, C., Sarzana, G. and Segré, E., *Arch. intern. physiol.*, 47: 245 (1938). p. 294, 302
Asada, K., *Biochem Z* , 142: 44 (1923); 144: 203 (1924). p. 342
Atkinson, H. V., Rapport, D. and Lusk, G., *J. Biol. Chem.*, 53: 155 (1922). p. 286, 287
Aylward, F. X., *see* Holt, L E., Jr
Azaretti, I , *see* Roffo, A. H
Bachrach, W. H., Bradley, W. B and Ivy, A C , *Am. J. Physiol* , 117: 203 (1936). p. 305
Baer, J. and Blum, L., *Arch. exptl. Path. Pharmakol* , 65: 1 (1911). p. 321
Balls, A. K., Matlack, M. B. and Tucker, I. W., *J. Biol. Chem.*, 122: 125 (1937). p. 318
Banks, A. and Hilditch, T. P., *Biochem. J.*, 26: 298 (1932). p 317
Barrett, H M., Best, C H. and Ridout, J. H , *J. Physiol.*, 93: 367 (1938). p. 300
Bauman, L., *J. Am. Med. Assoc.*, 90: 22 (1928) p. 309
Baumann, E. J and Holly, O. M., *Am J. Physiol.*, 75: 618 (1926). p. 341
Benedict, E M., *see* West, R
Berend, N , *see* Tangl, H.
Berg, W., *Z. mikroskop anat. Forsch.*, 1: 245 (1924). p. 296
Bernhard, K , *see* Flaschenträger, B.
Best, C. H , *see* Barrett, H. M
Best, C H. and associates, *J. Physiol* ,'75: 56, 405 (1932) p. 299
ibid., 97: 103 (1939). p. 299
Best, C. H. and Campbell, J , *J. Physiol.*, 86: 190 (1936). p. 297
ibid., 92: 91 (1938). p. 329
Best, C. H., Grant, R and Ridout, J. H., *J. Physiol.*, 86: 337 (1936). p. 299
Beumer, H , *Deut. med. Wochschr.*, 51: 230 (1925). p. 291, 292, 337
Biochem Z , 259: 469 (1933) p. 293, 337
Beumer, H. and Lehmann, F., *Z. ges. exptl. Med.*, 37: 274 (1923). p. 292
Bickenbach W. and Junkersdorf, P., *Arch. exptl. Path. Pharmakol.*, 132: 129 (1928). p. 305
Bickenbach, W. and Rupp, H., *Klin. Wochschr.*, 10: 63 (1931). p. 341
Birch, T. W., *J. Biol. Chem* , 124: 775 (1938). p. 319
Blackfan, K. D., *see* Gamble, J. L.
Blatherwick, N. R. and associates (Medlar, E. M., Bradshaw, P. J., Post, A. L. and Sawyer, S. D.), *Proc. Soc. Exptl. Biol. Med.*, 29: 345 (1931). p. 298
Blixenkrone-Møller, N., *Z. physiol. Chem.*, 252: 117 (1938a). p. 325
ibid., 252: 137 (1938b). p. 308
ibid., 253: 261 (1938c). p. 297, 327
Bloor, W. R., *J. Biol. Chem.*, 80: 443 (1928). p. 300
Proc. Soc. Exptl. Biol. Med., 36: 215 (1937a). p. 311
J. Biol. Chem., 119: 451 (1937b). p. 311
Bloor, W. R. and Snider, R. H., *J. Biol. Chem.*, 87: 399 (1930). p. 315, 316
Blum, L., *see* Baer, J.
Bonnet, R., *see* Terroine, E.-F.
Boyd, E. M. and Wilson, K, M., *J. Clin. Investigation*, 14: 7 (1935). p. 341
Bradley, W. B., *see* Bachrach, W. H.
Bradshaw, P. J., *see* Blatherwick, N. R.
Breusch, F., *see* Schoenheimer, R.
Brinkmann, E , *see* Toenniessen, E.
Broh-Kahn, R H , *see* Mirsky, I. A.
Brown, G. E. and Keith, N. M , *Arch. Internal Med.*, 33. 217 (1924). p. 309
Buchwald, K. W. and Cori, C. F., *Proc. Soc. Exptl Biol. Med.*, 28: 737 (1931). p. 311
Bürger, M. and Rückert, W., *Z. physiol. Chem.*, 196: 169 (1931). p. 290
Bürger, M. and Winterseel, W., *Z. physiol. Chem.*, 181: 255 (1929). p. 292

Burn, J. H. and Ling, H. W., *J. Physiol.*, 69: xix (1930). p. 297
Quart. J. Pharm. Pharmacol., 6: 31 (1933). p. 329
Burn, J. H. and Marks, H. P., *J. Physiol.*, 61: 497 (1926). p. 306
Burr, G. O. and Burr, M. M., *J. Biol. Chem.*, 82: 345 (1929). p. 318
ibid., 86: 587 (1930). p. 316
Burr, G. O., Burr, M. M. and Miller, E. S., *J. Biol. Chem.*, 97: 1 (1932). p. 318
Cahn, T., Inaug. Diss., Paris, 1928; Quoted by Needham, vol. II, p. 1169 (1931). p. 338, 340
Campbell, J., *see* Best, C. H.
Caspe, H. and S., *see* Funk, C.
Cathcart, E. P., *Biochem. J.*, 16: 747 (1922). p. 336
Cattoretti, E., *see* Dezani, S.
Chaikoff, I. L., *J. Biol. Chem*, 74: 203 (1927). p. 304
See also Entenman, C, Fries, B. A., Perlman, I.
Chaikoff, I. L. and Macleod, J. J. R., *Quart. J. Exptl. Physiol.*, 19: 291 (1929). p. 311
Chaikoff, I L. and Weber, J. J., *J. Biol. Chem.*, 76: 813 (1928). p. 305
Chamberlain, E. N., *see* Coope, R.
Chamberlin, P. E., Furgason, W. H. and Hall, V E., *J. Biol. Chem.*, 121: 599 (1937). p 334
Chambers, W. H. and associates (Kennard, M. A., Pollack, H. and Dann, M.), *J. Biol. Chem.*, 97: 525 (1932). p. 311
Changus, G. W., *see* Fries, B. A.
Channon, H. J., *Biochem. J.*, 19: 424 (1925). p. 292, 339
Channon, H. J., Irving, E. and Smith, J. A. B., *Biochem. J.*, 28: 1807 (1934). p. 316
Channon, H. J., Loach, J. V. and Tristram, G. R., *Biochem J*, 32. 1332 (1938). p. 299
Channon, H. J., Manifold, M. C. and Platt, A. P., *Biochem. J.*, 32: 969 (1938). p. 299
Chanutin, A., *see* Hortenstine, J. C.
Chidsey, J. L., *see* Dye, J. A.
Clark, D. E., *see* Dragstedt, L. R.
Clutterbuck, P. W. and Raper, H. S., *Biochem. J.*, 19: 385 (1925). p. 323
Collip, J P., *J. Biol. Chem*, 55: xxxviii (1923). p. 333
Coope, R., *J. Physiol.*, 60: 92 (1925). p 297
Coope, R. and Chamberlain, E. N., *J. Physiol.*, 60: 69 (1925) p 297
Coope, R and Mottram, V. H., *J. Physiol.*, 49: 23 (1914). p 297
Cori, C. F., *see* Buchwald, K. W, Cori, G. T.
Cori, G. T. and Cori, C. F., *J. Biol. Chem.*, 72: 615 (1927). p. 333
Coward, K. H., *see* Drummond, J. C.
Cramer, W., *see* Mottram, J. C
Cuthbertson, D. P, *Biochem. J.*, 19: 896 (1925). p. 310
Dakin, H. D., *J. Biol. Chem*, 4: 77 (1908a); 4: 91 (1908b). p 323
Dam, H., *Biochem. Z.*, 194: 188 (1928); 232: 269 (1931). p 291
ibid., 215: 475 (1929). p. 339, 340
D'Amato, L., *Biochem. Z.*, 69: 217 (1915). p 337
Daniels, A. L., *see* Mendel, L. B.
Dann, M., *see* Chambers, W. H.
Denis, W., *see* Folin, O.
Deuel, H. J., Jr and associates, *J Biol. Chem*, 109: 597 (1935); 116: 621 (1936). p. 325
ibid., 117: 119 (1937). p. 307
Deuel, H. J., Jr, Hallman, L. and Murray, S., *J. Biol. Chem*, 119: 257 (1937). p. 334
ibid., 124: 385 (1938). p. 332
Deuel, H. J, Jr., Wilson, H. E C. and Milhorat, A. T., *J. Biol. Chem.*, 74: 265 (1927). p 305
Dezani, S., *Giorn. accad med. Torino* [4S], 19: 149 (1913) p 291
Dezani, S. and Cattoretti, E., *Arch farm sper.*, 19: 1 (1915); *C. A.*, 9: 1338 (1915). p. 291
Dimits, L., *see* Fränkel, S.
Donaldson, L. R, *see* Norris, E R.
Donovan, P. B, *see* Dragstedt, L. R.
Dowler, V. B. and Mottram, V. H., *J. Physiol*, 52: 166 (1918). p. 306
Dragstedt, L. R. and associates (Donovan, P. B, Clark, D. E, Goodpasture, W. C. and Vermeulen, C) *Am. J. Physiol.*, 127: 755 (1939). p. 299
Drew, A. H., *see* Mottram, J. C.
Drummond, J. C., *see* Gregory, E.
Drummond, J. C. and Coward, K H., Lancet, 201, pt. 2. 698 (1921). p. 318
Drury, D. R., *see* McMaster, P. D
Duquenois, P., *see* Terroine, E.-F.
Dye, J. A. and Chidsey, J. L., *Am. J. Physiol*, 127 745 (1939). p 331
Dye, M., *see* Strouse, S.
Eagles, B. A., *see* Harding, V. J
Eaves, E. C., *J. Physiol.*, 40: 451 (1910). p. 316, 338
Eckstein, H. C., *J. Biol. Chem.*, 81: 613 (1929). p 287
ibid., 102: 591 (1933). p. 306
ibid., 125: 99, 107 (1938). p. 292
See also Tucker, H. F.
Ellis, G. W. and Gardner, J. A., *Proc. Roy. Soc. London*, B81: 129 (1909). p 291, 339
Ellis, N. R. and Zeller, J. H., *J. Biol. Chem.*, 89: 185 (1930). p. 341
Embden, G., *Beitr. chem. Physiol. Path.*, 6: 44 (1905). p. 306
Embden, G. and associates, *Beitr. chem. Physiol. Path.*, 8: 129 (1906); 11: 318 (1908). p. 322
Embden, G. and Marx, A., *Beitr. chem. Physiol. Path.*, 11: 318 (1908). p. 322
Embden, G., Salomon, H. and Schmidt, F., *Beitr. chem. Physiol. Path.*, 8: 129 (1906). p. 328
Emerique, L., *Bull. soc. chim. biol.*, 14: 305 (1932). p. 342
Entenman, C., Lorenz, F. W. and Chaikoff, I. L., *J. Biol. Chem.*, 133: 231 (1940). p. 340
Evans, E. A., Jr., *see* Rittenberg, D.
Evans, H. M. and Lepkovsky, S., *J. Biol. Chem.*, 96: 143 (1932). p. 318
Fales, H. L., *see* Holt, L. E.
Fiske, C. H. and Sokhey, S. S., *J. Biol. Chem.*, 63: 309 (1925). p. 335
Flaschenträger, —, *see* Lueg, W.
Flaschenträger, B. and Bernhard, K., *Z. physiol. Chem.*, 240: 19 (1936). p. 322
Folin, O. and Denis, W., *J. Biol. Chem.*, 21: 183 (1915). p. 334

Fox, F. W., *see* Gardner, J. A.
Fox, F. W. and Gardner, J. A., *Proc. Roy Soc. London*, B98: 76 (1925). p. 292
Frankel, E. M., *see* Ringer, A. I.
Fränkel, S. and Dimitz, L., *Wien. klin. Wochschr.*, 22: 1777 (1909). p. 311
Freiberg, I. K., *see* Greaves, J. D.
Friedemann, T. E., *see* Shaffer, P. A.
Friedmann, E., *Beitr. chem Physiol. Path.*, 11: 202 (1908). p 289
Fries, B. A. and associates (Ruben, S., Perlman, I. and Chaikoff, I. L.), *J. Biol. Chem.*, 123: 587 (1938). p. 302
Fries, B. A., Changus, G. W. and Chaikoff, I. L., *J Biol. Chem*, 132: 23 (1940). p. 294
Fukutomi, T., *see* Masai, Y.
Funk, C., Caspe, S. and Caspe, H., *Proc. Soc. Exptl Biol. Med*, 28: 816 (1931). p. 319
Furgason, W. H., *see* Chamberlin, P. E
Furusawa, K., *Proc. Roy. Soc. London*, B98: 65 (1925). p 310
Gage, S. H and Gage, S. P., *Science*, 28: 494 (1908). p 338
Anat. Record, 3: 203 (1909). p. 340
Gainsborough, H., *see* Gardner, J. A.
Gamble, J. L. and Blackfan, K. D., *J Biol. Chem*, 42: 401 (1920). p 292
Gardner, J. A., *see* Ellis, G. W., Fox, F W
Gardner, J. A. and Fox, F. W., *Proc. Roy. Soc. London*, B92. 358 (1921); B93· 486 (1922). p. 292
Gardner, J. A. and Gainsborough, H., *Quart. J Med*, 23: 465 (1930). p 337
Gardner, J. A. and Lander, P. E., *Proc Roy Soc. London*, B87: 229 (1914). p. 291
Geelmuyden, H. C., *Norsk Mag. Laegevidenskap*, 81: 479 (1920a); *J. Am. Med. Assoc.*, 74: 1684 (1920a). p 304
Acta Med. Scand., 53· 381 (1920b). p. 295
ibid., 54: 51 (1921). p 303
Gemmill, C. L. and Holmes, E. G, *Biochem J.*, 29: 338 (1935). p. 307
Goldberg, M., *Am. Rev. Tuberc.*, 8· 245 (1923). p 295
Goodpasture, W. C., *see* Dragstedt, L. R.
Gräfe, E, *Ergeb. Physiol*, 21: 197 (1923) p. 308
Gräfe, E. and Weissmann, A, *Deut. Arch klin Med*, 143: 350 (1924) p. 336
Graham, C. E. and Griffith, W. H., *Proc Soc. Exptl. Biol Med*, 28. 756 (1931). p 319
Graham, S. G., *see* Imrie, C. G.
Grant, R., *see* Best, C. H.
Grayman, I., *see* Nelson, N.
Greaves, J. D., Freiberg, I. K. and Johns, H. E, *J Biol Chem*, 133: 243 (1940). p 297
Greene, C. W, *J. Biol. Chem.*, 39· 435 (1919); 48: 59 (1921). p 309
Gregory, E. and Drummond, J. C, *Z. Vitaminforsch*, 1· 257 (1932); *C. A*, 27: 2982 (1933). p 319
Greisheimer, E. M., *J. Nutrition*, 4: 411 (1931) p. 306
Grey, E. C., *Biochem. J.*, 7: 148 (1913). p. 320
Griffith, W. H., *see* Graham, C. E.
Griffith, W. H. and Wade, N. J., *J Biol Chem*, 131: 567 (1939) p 299
Haarmann, W. and Schroeder, E., *Biochem Z*, 296· 35 (1938). p. 308
Haggard, H. W, *see* Henderson, Y.
Hahn, L A and Hevesy, G. C., *Biochem. J.*, 32: 342 (1938). p. 294
Hall, V. E., *see* Chamberlin, P. E.
Halliday, N., *J. Nutrition*, 16: 285 (1938). p. 300
Hallman, L. F., *see* Deuel, H. J., Jr.
Hanes, F. M., *J. Exptl Med.*, 16: 512 (1912). p 339
Hannon, R. R. and McCann, W. S., *Bull. Johns Hopkins Hosp*, 33: 128 (1922). p. 331
Hansen, C., *see* Henriques, V.
Harding, V. J. and coworkers (Allin, K. D., Eagles, B. A. and Van Wyck, H. B.), *J. Biol. Chem.*, 63: 37 (1925). p. 336
Hartley, P., *J. Physiol*, 38: 353 (1909). p 316
Hartley, P. and Mavrogordato, A., *J. Path Bact*, 12: 371 (1908) p. 295
Haven, F. L., Abst. 3rd Internat. Cancer Cong, Atlantic City, 120 (1939). p 294
Hawley, E. E., *Am J. Physiol.*, 101: 185 (1932). p. 305
Hawley, E. E. and Murlin, J. R., *Am. J. Physiol*, 75: 107 (1925). p 333
ibid., 101: 51 (1932). p. 307
Hawley, E. E., Johnson, C. W. and Murlin, J. R, *Am. J. Physiol.*, 93: 656 (1930). p. 307
J. Nutrition, 6: 523 (1933). p. 307, 334
Heffter, A., *Arch. exptl. Path. Pharmakol*, 28: 97 (1891). p. 300
Heller, H., *Acta Med. Scand.*, 90: 489 (1936). p. 307
Hel'man, R. M., *see* Koldaev, B. M.
Henderson, L. J. and Palmer, W. W., *J. Biol. Chem.*, 13: 393 (1913). p. 335
Henderson, Y. and Haggard, H. W., *J. Biol. Chem.*, 63: lxix (1925). p. 310
Henriques, V. and Hansen, C., *Skand. Arch. Physiol.*, 11: 151 (1901). p. 315
Hentschel, H., *Z. Kinderheilk.*, 52: 623 (1932). p. 342
Hepner, J. and Wagner, O., *Biochem. Z.*, 189: 322 (1927). p. 298
Hevesy, G. C., *see* Hahn, L. A.
Hevesy, G. C., Levi, H. B. and Rebbe, O. H., *Biochem. J.*, 32: 2147 (1938). p. 339
Hilditch, T. P., *see* Banks, A.
Hilditch, T. P. and Longenecker, H. E., *Biochem. J.*, 31: 1805 (1937). p. 317
Hilditch, T. P. and Pedelty, W. H., *Biochem. J.*, 34: 40 (1940). p. 290
Himwich, H. E., *Yale J. Biol. Med.*, 4: 259 (1932). p. 290
See also Meyerhof, O.
Holly, O. M., *see* Baumann, E. J.
Holmes, E. G., *see* Gemmill, C. L.
Holt, L. E. and Fales, H. L., *Am. J. Diseases Children*, 25: 247 (1923). p. 336
Holt, L. E., Jr., Aylward, F. X. and Timbus, H. G., *Acta Paediat.*, 22: 425 (1937). p. 298
Hoppe-Seyler, F., *Z. physiol. Chem.*, 3: 351 (1879). p. 288
Hortenstine, J. C., Chanutin, A. and Ludewig, S., *J. Biol. Chem.*, 125: 455 (1938). p. 299
Hotta, K., *Z. physiol. Chem.*, 128: 85 (1923); 136: 1 (1924). p. 342
Houssay, B. A., *Klin. Wochschr.*, 11: 1529 (1932). p. 304
Hubbard, R. S., *J. Biol. Chem.*, 55: 357 (1923). p. 334

Hubbard, R. S. and Nicholson, S. T., *J. Biol. Chem.*, 53: 209 (1922). p. 331
Hubbard, R. S. and Wright, F. R., *J. Biol. Chem.*, 50: 361 (1922). p. 314
Hume, E. M. and associates (Nunn, L. C. A., Smedley-MacLean, I. and Smith, H. H.), *Biochem. J.*, 32: 2162 (1938) p. 319
Hume, E. M. and Smith, H. H., *Biochem. J.*, 25: 300 (1931) p. 319
Hynd, A. and Rotter, D. L., *Biochem. J.*, 26: 578, 1633 (1932) p. 297
Imrie, C. G., *J. Path. Bact.*, 19: 245 (1914) p. 295, 316
Imrie, C. G. and Graham, S. G., *J. Biol. Chem.*, 44: 243 (1920). p. 341
Irving, E., *see* Channon, H. J.
Ivy, A. C., *see* Bachrach, W. H.
Jaeckle, H., *Z. physiol. Chem.*, 36: 53 (1902). p. 341
Jaffé, R., *Arch. exptl. Path. Pharmakol.*, 132: 84 (1928). p. 318
Joannovics, G. and Pick, E. P., *Wien. klin. Wochschr.*, 23: 573 (1910) p. 296, 315
Johns, H. E., *see* Greaves, J. D.
Johnson, C. W., *see* Hawley, E. E.
Johnston, M. W., *see* Newburgh, L. H.
Jonas, L., *see* Ringer, A. I.
Jost, H., *Z. physiol. Chem.*, 197: 90 (1931). p. 306
Jowett, M. and Quastel, J. H., *Biochem. J.*, 29: 2143, 2159, 2181 (1935). p. 325
Junkersdorf, P., *see* Bickenbach, W.
Kahn, M., *Am. J. Med. Sci.*, 166: 826 (1923). p. 328
Kaufmann, C. and Mühlbock, O., *Z. ges. exptl. Med.*, 89: 200 (1933). p. 337
Kay, H. D. and Raper, H. S., *Biochem. J.*, 16: 465 (1922). p. 323
ibid., 18: 153 (1924). p. 324
Keefer, C. S., Perlzweig, W. A. and McCann, W. S., *Bull. Johns Hopkins Hosp.*, 35: 265 (1924). p. 329
Keith, N. M., *see* Brown, G. E.
Kennard, M. A., *see* Chambers, W. H.
Kennaway, E. L., *Biochem. J.*, 8: 355 (1914). p. 335
Kennaway, E. L. and Leathes, J. B., *Lancet*, 1: 95 (1909). p. 315, 316
King, E. J., *see* Tait, H.
Klenk, E., *Z. physiol. Chem.*, 185: 169 (1929). p. 290
Klenk, E. and Schönebeck, O., *Z. physiol. Chem.*, 209: 112 (1932). p. 316
Knoop, F., *Beitr. chem. Physiol. Path.*, 6: 150 (1905). p. 322
Knudson, A., *see* Randles, F. S.
Koldaev, B. M. and Hel'man, R. M., *Biochem. J.* (Ukraine), 10: 868 (1937); *C. A.*, 32: 6316 (1938). p. 311
Kreidl, A. and associates, *Zentr. Physiol.*, 24: 2, 54 (1910) p. 340
Krogh, A. and Lindhard, J., *Biochem. J.*, 14: 290 (1920) p. 305
Kugler, O. E., *Am. J. Physiol.*, 115: 287 (1936). p. 338
Kurnaev, E. F., *see* Pentegov, B. P.
Kusui, K., *Z. physiol. Chem.*, 181: 101 (1929). p. 291, 340
ibid., 187: 210 (1930). p. 340
Lander, P. E., *see* Gardner, J. A.
Lattes, L., *Biochem. Z.*, 20: 215 (1909). p. 306
Lawaczeck, H., *Z. physiol. Chem.*, 125: 229 (1923). p. 342
Leathes, J. B., "Problems in Animal Metabolism," London, J. Murray, 1906 p. 288
Arch. exptl. Path. Pharmakol., Supp. Bd., "Schmiedeberg Festschrift," 1908 327. p. 289
Leathes, J. B. and associates, *Lancet*, 1: 95 (1909a); *J. Physiol.*, 38: xxxviii (1909b) p. 297
Leathes, J. B. and Meyer-Wedell, L., *J. Physiol.*, 38: xxxviii (1909) p. 315
Leathes, J. B. and Raper, H. S., "The Fats," 2d ed., p. 163, New York, Longmans, Green and Co., 1925 p. 295
Le Breton, E., *Bull. soc. chim. biol.*, 3: 539 (1921). p. 339
Lecoq, R., *Bull. soc. chim. biol.*, 15: 1498 (1933). p. 342
Lecoq, R. and Savore, J., *Bull. soc. chim. biol.*, 15: 1508 (1933). p. 342
Lee, J. van der, *see* Verkade, P. E.
Lehmann, F., *see* Beumer, H.
Lepkovsky, S., *see* Evans, H. M.
Levi, H. B., *see* Hevesy, G. C.
Levine, H. and Smith, A. H., *J. Biol. Chem.*, 72: 223 (1927) p. 336
Liang, B. and Wacker, L., *Biochem. Z.*, 164: 371 (1925). p. 342
Lindhard, J., *see* Krogh, A.
Ling, H. W., *see* Burn, J. H.
Ling, S. M., *see* Wiersuchowski, M.
Loach, J. V., *see* Channon, H. J.
Loew, O., *Biol. Zentr.*, 11: 269 (1891). p. 300
London, E. S., *Ergeb. Physiol.*, 26: 320 (1928). p. 294, 315
Long, C. N. H., *see* Shipley, R. A.
Longenecker, H. E., *J. Biol. Chem.*, 128: 645 (1939). p. 288
See also Hilditch, T. P.
Lorenz, F. W., *see* Entenman, C.
Lovern, J. A., *Biochem. J.*, 30: 2023 (1936). p. 317
Lubrzynska, E. and Smedley, I., *Biochem. J.*, 7: 375 (1913). p. 289
Ludewig, S., *see* Hortenstine, J. C.
Lueg, W. and Flaschenträger, —, *Klin. Wochschr.*, 4: 694 (1925). p. 305
Lukens, F. D. W., *see* Stadie, W. C.
Lundin, H., *J. Metabolic Research*, 4: 151 (1923). p. 329
Lusk, G., *Z. Biol.*, 42: 31 (1901). p. 305, 321
Am. J. Physiol., 22: 163 (1908). p. 306, 321
See also Atkinson, H. V., Murlin, J. R., Ray, W. E.
Lyon, D. M., Robson, W. and White, A. C., *Brit. Med. J.*, 1925: (1) 207. p. 329
McAmis, A. J., Anderson, W. E. and Mendel, L. B., *J. Biol. Chem.*, 82: 247 (1929). p. 318
MacCallum, A. B., *Can. Med. Assoc. J.*, 22: 3 (1930). p. 332, 334
McCann, W. S., *see* Hannon, R. R., Keefer, C. S.
McDermott, T. S., *see* Ray, W. E.

MacKay, E. M. and Wick, A N , *Am. J. Physiol* , 126: 753 (1939). p. 330
MacLean, I. S., *see* Smedley-MacLean, I.
Macleod, J. J. R., *see* Anderson, I. A., Chaikoff, I. L.
McMaster, P. D., *J. Exptl. Med.*, 40: 25 (1924). p. 337
McMaster, P. D. and Drury, D. R., *Proc. Soc. Exptl Biol. Med* , 25: 151 (1927). p. 316
Magnus-Levy, A , *Ergeb inn. Med.*, 1: 352 (1908). p. 335
Maignon, F., *Compt rend. soc. biol.*, 86: 197 (1922) p. 334
Bull. soc. chim. biol., 11: 943 (1929). p. 306
Manifold, M. C., *see* Channon, H J.
Marks, H. P., *see* Burn, J. H
Marriott, W. M , *J. Biol. Chem.*, 18: 507 (1914). p. 328
Marsh, P. J., *see* Newburgh, L. H.
Marx, A., *see* Embden, G
Masai, Y. and Fukutomi, T., *J. Biochem. (Japan)*, 2. 271 (1923). p. 338
Matlack, M. B., *see* Balls, A. K.
Mavrogordato, A., *see* Hartley, P.
Mayer, A. and Schaeffer, G., *J. physiol. path. gén.*, 16: 325 (1914). p. 296, 300
Mazza, F. P., *Arch. sci biol (Italy)*, 22: 307 (1936). p. 322
Medlar, E. M., *see* Blatherwick, N. R
Mendel, L. B., *see* Anderson, W. E , McAmis, A. J
Mendel, L. B and Daniels, A L , *J Biol Chem.*, 13: 71 (1912). p. 341
Menschick, W , *see* Page, I. H.
Mentov, Y. N , *see* Pentegov, B. P.
Meyer-Wedell, L., *see* Leathes, J. B.
Meyerhof, O., *Arch. ges Physiol (Pflügers)*, 199: 531 (1923). p 311
Meyerhof, O and Himwich, H E., *Arch. ges Physiol.*, 205: 415 (1924) p 311
Miescher, F., "Histochemischen und physiologischen Arbeiten," Leipzig, Vogel, 1897. p. 309
Milhorat, A. T., *see* Deuel, H J., Jr
Miller, E. S., *see* Burr, G. O.
Mirsky, I. A., *Am. J. Physiol.*, 115: 424 (1936). p. 297
See also Nelson, N.
Mirsky, I. A and Broh-Kahn, R. H., *Am. J. Physiol.*, 120· 446 (1937). p. 329
Mitchell, H H., *J. Nutrition*, 6: 473 (1933). p. 307, 308
Modern, F. S., *J. Metabolic Research*, 4· 177 (1923). p 329
Monaghan, B. R , *J. Biol. Chem* , 98: 21 (1932) p. 342
Mottram, J C., Cramer, W. and Drew, A. H., *Brit. J. Exptl. Path.*, 3: 179 (1922). p. 342
Mottram, V H., *J Physiol* , 45: 363 (1912) p 315
See also Coope, R , Dowler, V B.
Mühlbock, O , *see* Kaufmann, C.
Mueller, J. H , *J Biol. Chem.*, 21: 23 (1915). p. 339
Müller, P., *Verh. schweiz Physiol* , 11: 8 (1937); *C. A* , 32: 6308 (1938). p. 321
Murlin, J. R., *Am J Obstet. Diseases Women, Children*, 75: 913 (1917) p. 340
See also Hawley, E E.
Murlin, J R and Lusk, G., *J Biol Chem* , 22: 15 (1915). p. 336
Murray, S , *see* Deuel, H J , Jr
Murrell, F C., *see* Wesson, L G.
Nagayama, T. and Tagaya, T , *J Biochem. (Japan)*, 11: 225 (1929). p. 343
Needham, J., "Chemical Embryology," Cambridge, University Press, 1931. p. 337, 339, 340
Nelson, N., Grayman, I. and Mirsky, I. A , *J. Biol. Chem.*, 132: 711 (1940). p. 330
Neuberg, C. and Arinstein, B., *Biochem. Z.*, 117: 269 (1921). p. 289
Newburgh, L. H. and Johnston, M. W , *J. Clin. Investigation*, 8: 197 (1930). p. 309
Newburgh, L. H. and Marsh, P J , *Arch. Internal Med.*, 27: 699 (1921). p. 334
Nicholson, S. T., *see* Hubbard, R. S.
Norris, E R and Donaldson, L. R , *Am. J. Physiol.*, 129: 214 (1940). p. 299
Nunn, L. C. A., *see* Hume, E. M.
Nunn, L. C. A. and Smedley-MacLean, I., *Biochem. J.*, 32: 2178 (1938). p. 319
Osaki, J., *Biochem Z.*, 189· 233 (1927). p 320
Page, I H. and Menschick, W., *J. Biol. Chem.*, 97: 359 (1932). p 336
Page, I. H. and Young, F. G., *Biochem. J.*, 26: 1528 (1932). p. 306
Palmer, W. W., *see* Henderson, L. J.
Paton, D. N., *J. Physiol.*, 19: 167 (1896). p 296, 306
Pearson, L. K. and Raper, H S., *Biochem. J.*, 21: 875 (1927). p. 289
Pedelty, W. H., *see* Hilditch, T P.
Pentegov, B. P., Mentov, Y N and Kurnaev, E. F., *Bull. Pacific Sci. Fishery Research Sta.* (Vladivostok) 2: Pt. 1, 3 (1928); *C. A.*, 23: 2502 (1929). p 309
Perlman, I., *see* Fries, B. A.
Perlman, I. and Chaikoff, I. L., *J. Biol. Chem.*, 127: 211 (1939). p. 299
Perlzweig, W. A., *see* Keefer, C S.
Petren, K., *J. Biol. Chem* , 61: 355 (1924) p 334
Pfeiffer, G., *Biochem Z.*, 222: 214 (1930). p. 291, 293
Pick, E. P., *see* Joannovics, G.
Platt, A. P., *see* Channon, H J.
Platz, B. R., *see* Schneider, H.
Plimmer, R. H. A. and Scott, F. H., *J. Physiol.*, 38: 247 (1909) p. 338
Pollack, H., *see* Chambers, W. H.
Post, A. L., *see* Blatherwick, N. R.
Quastel, J. H., *see* Jowett, M
Randles, F. S and Knudson, A , *J Biol Chem* , 66: 459 (1925). p. 291
Raper, H. S , *J Physiol.*, 32: 216 (1905) p 288
J. Chem. Soc , 91: 1831 (1907a). p. 288
J. Physiol., 35: xxiv (1907b). p. 289
J. Biol. Chem., 14: 117 (1913). p. 316
See also Clutterbuck, P W , Kay, H. D , Leathes, J B , Pearson, L. K.

Raper, H. S. and Smith, E. C., *J. Physiol.*, **60**: 41 (1925). p. 290, 306
ibid., 62: 17 (1926). p. 328, 333
Raper, H. S. and Wayne, E. J., *Biochem. J.*, **22**: 188 (1928). p. 324
Rapport, D., *see* Atkinson, H. V.
Raudnitz, R W , *Z. Biol.*, **24**: 423 (1888). p 341
Ray, W. E., McDermott, T. S. and Lusk, G., *Am. J. Physiol.*, **3**: 139 (1899). p. 295
Rebbe, O H , *see* Hevesy, G. C.
Reichel, L. and Schmid, O., *Biochem Z.*, **300**: 274 (1939). p 289
Remington, R E., *J. Nutrition*, **16**: 417 (1938). p. 329
Riddle, O , *Am. J. Physiol.*, **41**: 409 (1916). p. 338
Ridout, J. H , *see* Barrett, H. M., Best, C. H.
Rietti, C. T., *Compt rend. soc. biol.*, **117**: 57 (1934). p. 329
Ringer, A. I , *J Biol Chem.*, **12**: 511 (1912). p. 321, 322
ibid., **14**: 43 (1913). p 306, 321, 322
Ringer, A. I., Frankel, E. M. and Jonas, L., *J. Biol. Chem.*, **14**: 539 (1913). p. 321
Rittenberg, D., *see* Schoenheimer, R
Rittenberg, D. and Schoenheimer, R , *J. Biol Chem.*, **121**: 235 (1937). p. 293
Rittenberg, D , Schoenheimer, R and Evans, E. A., Jr , *J. Biol Chem.*, **120**: 503 (1937). p. 322
Robson, W , *see* Lyon, D M.
Roffo, A. H. and Azaretti, I , *Bol. inst. med. exptl.*, **2**: 629 (1926); *C. A.*, **21**: 2924 (1927). p. 339, 340
Rose, W. C., *J. Pharmacol.*, **24**: 123, 147 (1924). p 321
Rose, W. C and associates, *J. Pharmacol.*, **25**: 59, 65 (1925). p. 321
Rosenfeld, G., *Biochem Z.*, **209**: 312 (1929). p 295
Rosenthal, F., *Klin. Wochschr.*, **10**. 700 (1931). p. 306
Rotter, D L., *see* Hynd, A.
Ruben, S., *see* Fries, B. A.
Ruckert, W., *see* Bürger, M.
Rupp, H., *see* Bickenbach, W.
Russell, J. A., *Am J. Physiol.*, **121**: 755 (1938). p. 329
Salomon, H , *see* Embden, G.
Sarzana, G , *see* Artom, C.
Savore, J , *see* Lecoq, R.
Sawyer, S D., *see* Blatherwick, N. R.
Schaber, H , *see* Thannhauser, S. J.
Schaeffer, G., *see* Mayer, A.
Schmid, O , *see* Reichel, L.
Schmidt, F , *see* Embden, G.
Schneider, H., Steenbock, H. and Platz, B R , *J. Biol Chem* , **132**: 539 (1940). p. 318
Schonebeck, O , *see* Klenk, E
Schoenheimer, R., *Z. physiol* chem , **180**: 1 (1929). p. 293
Z klin Med., **123**: 749 (1933). p 337
See also Rittenberg, D , Stetten, D , Jr
Schoenheimer, R and Breusch, F , *J Biol Chem* , **103**: 439 (1933). p. 293
Schoenheimer, R and Rittenberg, D , *J Biol. Chem* , **113** 505 (1936). p 317
Schoenheimer, R and Sperry, W M , *J Biol Chem* , **107**. 1 (1934) p 337
Schroeder, E , *see* Haarmann, W
Scott, F H., *see* Plimmer, R. H. A.
Segré, E , *see* Artom, C.
Shaffer, P. A., *J Biol. Chem.*, **47**: 433 (1921); *Harvey Lectures*, **18**: 105 (1923). p. 331
Shaffer, P. A. and Friedemann, T. E , *J. Biol. Chem* , **61**: 585 (1924). p 331
Sharpe, J S , *Biochem. J.*, **16**: 486 (1922). p 342
Shibata, N , *Biochem. Z.*, **37**: 345 (1911). p 295
Shipley, R A. and Long, C. N. H , *Biochem. J.*, **32**. 1242 (1938). p. 297, 329
Sinclair, R G., *J. Biol. Chem* , **82**: 117 (1929). p. 300, 301, 315
ibid., **92**: 245 (1931). p. 301
ibid , **96**: 103 (1932). p 317, 319
Am. J. Physiol., **103**: 73 (1933). p. 341
Physiol Revs., **14**: 351 (1934). p. 317
J. Biol Chem., **111**: 515 (1935). p. 294
ibid , **115**: 211 (1936). p. 301
J. Nutrition, **19**: 131 (1940). p. 320
Slemons, J. M. and Stander, H. J , *Bull. Johns Hopkins Hosp.*, **34**: 7 (1923). p. 340
Smedley, I , *J. Chem. Soc.*, **99**: 1627 (1911). p. 288
See also Smedley-MacLean, I.
Smedley-MacLean, I., *see* Hume, E. M., Lubrzynska, E., Nunn, L. C. A.
Smith, A. H., *see* Levine, H.
Smith, E. C , *see* Raper, H. S.
Smith H. G., *J. Biol Chem* , **103**: 531 (1933). p. 320
Smith, H. H., *see* Hume, E. M
Smith, J A. B , *see* Channon, H. J.
Snider, R. H., *see* Bloor, W R
Sokhey, S. S., *see* Fiske, C. H.
Soskin, S , *Biochem. J.*, **23**: 1385 (1929); *J. Nutrition*, **3**: 99 (1930). p. 305
Sperry, W. M , *see* Schoenheimer, R.
Sperry, W. M. and Stoyanoff, V. A., *J. Nutrition*, **9**: 157 (1935). p. 337
Stadie, W. C., Zapp, J. A., Jr. and Lukens, F. D. W., *J. Biol. Chem.*, **132**: 423 (1940). p. 325
Stander, H. J., *see* Slemons, J. M.
Steenbock, H., *see* Schneider, H.
Stetten, D , Jr. and Schoenheimer, R., *J. Biol. Chem.*, **133**: 329, 347 (1940). p. 291
Stohr, R., *Z. physiol. chem.*, **217**: 141 (1933a); **220**: 27 (1933b). p. 304
Stoyanoff, V. A., *see* Sperry, W. M.
Strouse, S., *see* Wang, C. C.
Strouse, S., Wang, C. C. and Dye, M., *J. Am. Med. Assoc.*, **82**: 2111 (1924a); *Arch. Internal Med.*, **34**: 275 (1924b) p. 308

Szent-Györgyi, A. v., *Biochem. Z.*, **146**: 245 (1924). p. 311
Tagaya, T., *see* Nagayama, T.
Tait, H. and King, E. J., *Biochem. J.*, **30**: 285 (1936). p. 311
Takao, T., *Biochem. Z.*, **172**: 272 (1926). p. 336
Tangl, H. and Berend, N., *Biochem. Z.*, **220**: 234 (1930) p. 317
Terashima, T., *Japan. J. Gastroenterol.*, **9**: 263 (1937); *C. A.*, **32**: 5892 (1938). p 328
Terroine, E.-F., *Ann. sci. nat Zool.* [10], **4**: 92 (1920). p. 295
Terroine, E.-F. and Weill, J., *J. physiol. path. gén.*, **15**: 549 (1913). p 290
Terroine, E.-F., Bonnet, R. and Duquenois, P., *Bull. soc. chim. biol.*, **9**: 597 (1927). p. 302
Thannhauser, S. J. and Schaber, H., *Z. physiol. Chem.*, **127**: 278 (1923). p. 291, 339
Theis, E. R., *J. Biol. Chem.*, **76**: 107 (1928a); **77**: 75 (1928b). p. 300
Thiemich, M., *Jahrb. Kinderheilk.*, **61**: 174 (1905). p. 341
Thunberg, T., *Skand. Arch. Physiol.*, **24**: 90 (1911). p. 300
Timbus, H. G., *see* Holt, L. E., Jr.
Toenniessen, E. and Brinkmann, E., *Z. physiol. Chem.*, **252**: 169 (1938). p. 328
Tristram, G. R., *see* Channon, H. J.
Tucker, H. F. and Eckstein, H. C., *J. Biol Chem*, **126**: 117 (1938). p. 299
Tucker, I. W., *see* Balls, A. K.
Turpeinen, O., *Proc Soc. Exptl Biol Med*, **37**: 37 (1937) p. 318
Valla, S., *Bull. soc. chim. biol.*, **17**: 1715 (1935). p. 292
van der Lee, J., *see* Lee, J. van der
Van Wyck, H B, *see* Harding, V. J.
Verkade, P. E. and Lee, J van der, *Biochem. J.*, **28**: 31 (1934a); *Z. physiol. Chem.*, **225**: 230 (1934b); **227**: 213 (1934c). p 321
Vermeulen, C, *see* Dragstedt, L. R.
Wacker, L., *see* Liang, B
Wade, N. J., *see* Griffiths, W. H.
Wagner, O, *see* Hepner, J.
Wang, C. C., *see* Strouse, S.
Wang, C C and Strouse, S., *Arch Internal Med*, **36**: 397 (1925) p. 309, 319
Wayne, E J., *see* Raper, H. S
Weber, J. J., *see* Chaikoff, I. L.
Weill, J., *see* Terroine, E -F.
Weissmann, A., *see* Gräfe, E.
Welch, A D. and Welch, M. S, *Proc. Soc. Exptl. Biol. Med.*, **39**: 7 (1938). p. 299
Wertheimer, E., *Arch. ges Physiol.*, **213**: 262 (1926). p. 298
Arch. exptl. Path Pharmakol, **160**: 177 (1931) p 298
Wesson, L. G, *Bull. Johns Hopkins Hosp*, **38**: 237 (1926). p 341
J Biol. Chem., **73**: 507 (1927). p 319
Wesson, L. G. and Murrell, F. C, *J. Biol. Chem.*, **102**: 303 (1933). p. 319
West, E. S., *J. Biol. Chem*, **66**: 63 (1925); **74**: 561 (1927) p. 331
West, R and Benedict, E. M., *J. Biol. Chem.*, **66**: 139 (1925) p. 324
White, A C., *see* Lyon, D M.
Wick, A N, *see* MacKay, E M.
Wierzuchowski, M and Ling, S M, *J Biol Chem.*, **64**: 697 (1925). p. 290
Wigglesworth, V. B., *Biochem. J.*, **18**: 1203 (1924). p. 333, 335
Wilder, R M., *J. Am. Med Assoc.*, **78**: 1878 (1922). p. 331
Wilson, H E C., *see* Deuel, H. J, Jr.
Wilson K M., *see* Boyd, E. M.
Winfield, G, *J Physiol*, **49**: 171 (1915). p. 310
Wintersceel, W *see* Bürger, M.
Woodyatt, R. T., *Arch. Internal Med.*, **28**: 125 (1921). p. 331
Wright, F. R., *see* Hubbard, R. S.
Young, F G., *see* Page, I H.
Zapp, J. A., Jr, *see* Stadie, W. C.
Zeller, J. H., *see* Ellis, N. R.

Chapter VI

The Lipids of Secretions and Excretions

In this chapter is included a discussion of the lipids in the variety of liquids and solids which are produced by the tissues of living organisms and removed from the cells producing them. Some of this material is useful to the organism; some consists of substances which are no longer useful and are being discarded. The separation of useful from waste substances is often not complete, small amounts of waste material appearing in the useful secretions, and vice versa.

Secretions

Food for the embryo and young organism

Milk, eggs, and the material which passes through the placenta from the mother to the fetus are for the nourishment of the growing embryo. The store of food in plant seeds is for the growth of the young plant. To what extent are these food materials specially adapted to the needs of the rapidly growing organisms and to what extent do they represent the food of the adult parents and therefore material to which the young organism must adapt itself? These questions can be answered in part. For a general discussion of this subject, the reader is referred to Joseph Needham's monograph on chemical embryology (1931).

The proteins of milk and eggs and also of seeds are special products not to be found in the bodies of the animals or the plants. The carbohydrate of milk (lactose) is found only in milk. The specific constituent of lactose, the hexose galactose, is found in the nervous system of adults in the cerebrosides of brain and nerves. The cerebrosides are present in much smaller amounts in the newborn animal than in the adult, and the provision of the sugar galactose in the milk is probably to be referred to the needs of the developing nervous system.

To what extent are the lipids of milk peculiar to the milk and to what extent do they represent the fat of the mother's food which has been transferred to the milk with little change? In the milk fat we recognize oleic, palmitic, and stearic acids, fatty acids universally found in natural fats. The animal and the plant can form these fatty acids readily from carbohydrate. They constitute most of the fatty acids of milk; and, because of their universal distribution in the food and body of the animal,

it is hardly ever possible to say whether they come from the food or are specially manufactured. The lower fatty acids (butyric, caproic, etc.) which are found in milk are undoubtedly special products because they do not occur in significant amounts elsewhere in the body of the animal producing them. Their significance is not known. Studies outlined below indicate that the fat of the mother's food may be transferred with greater or less directness to the milk. In carnivorous and omnivorous animals, the transfer is often immediate; but in herbivorous animals it may take a relatively long time, and the evidence indicates that it undergoes physical and chemical transformation before it appears in the milk.

The fat of eggs can be readily shown to originate largely in the food of the hen.

The case of plant seeds is simpler. All the fat present has come from the elemental carbon dioxide and water by way of carbohydrate, and presumably the stored fat is always a product as well adapted to the needs of the young plant as the conditions of the environment allow. The large proportion of mixed glycerides in plant and animal products intended for consumption by embryonic or young life, as noted by Bhattacharya and Hilditch (1930), is of unknown significance. Because of their possible optical activity, because the phospholipids are also mixed glycerides and optically active, and because, in general, products intimately associated with life processes are optically active, the mixed nature of the glycerides may be assumed to be an adaptation to their future use. Physical factors, such as the desirability of conservation in liquid form under various environmental conditions, may also be important.

The placenta. It has been a much disputed point whether the lipids in the blood of the mother can be transferred across the placenta to the fetus, and the consensus of opinion and evidence up to recently has been against such a transfer. Recent work, however, indicates that in the case of some lipids and in some animals such a transfer is possible and may be extensive. Since the nature of the placenta is widely different in different species, it is probable that this difference is responsible for the varying results. A summary of the structure of the placenta in different animals and of the passage of various substances through it is given by Needham (1931).

In general, soluble, diffusible substances pass through the placenta freely, whereas insoluble, non-diffusible, large-molecular substances do not pass. Since the fatty substances are mostly insoluble and non-diffusible, it was assumed that they would not pass the placenta, and most of the evidence until recently supported the assumption (Thiemich, 1905), (see also, discussion of the blood lipids in pregnancy, Chapter III, p. 150). However, Bickenbach and Rupp (1933) have demonstrated that

the placenta of the rabbit is permeable to the highly unsaturated acids of linseed oil, and Sinclair (1933) has shown beyond question that the placenta of the rat is permeable to the highly unsaturated acids of cod liver oil. Sinclair notes, however, that the placentas of the rabbit and the rat are of the simplest type, only the endothelium of the fetal blood vessels separating the fetal from the maternal blood—a fact which made it unwise to conclude without further proof that the more complicated human placenta is permeable to fat. Some of the needed proof was supplied by Boyd and Wilson (1935), who were able to demonstrate that the human fetus received phospholipid and free cholesterol, also at times cholesterol esters, from the mother's blood and from the placental circulation, and that the amount of material transferred was about 50 grams per day, 75 per cent of which was phospholipid and the rest about equally cholesterol and cholesterol esters. In work on rabbits, Boyd (1935) brought evidence to show that the placenta acted somewhat as a secreting mechanism actively engaged in the transfer of lipid rather than as a membrane permeable to the lipid. McConnell and Sinclair (1937) showed that when elaidic acid was fed to the mothers the newborn young contained 16 per cent of their liver phospholipid fatty acids and 11 per cent of their whole body fatty acids as elaidic acid. After 10 days' suckl.ng, the elaidic acid in the body fatty acids had risen to 61 per cent and in the liver phospholipid fatty acids to 27 per cent.

Milk. Milk constitutes the sole source of maintenance and growth of the young mammal from birth over a considerable period of time and should therefore provide most of the answers to the problem of what it takes to keep the young animal alive and developing. When the mother has access to proper food, milk is probably as nearly a complete food as it is possible to get. In spite of the enormous amount of study which has been devoted to milk, there is very little information available regarding its lipid constituents in relation to the needs of the young animal. Many investigations have been made on the fat of milk, but almost entirely from the commercial point of view; practically none of it regards the peculiar usefulness, if any, of the milk fat and other lipids to the young animal.

Because of its wide use as human food, cow's milk has been much more extensively studied than the milk of any other animal and most of the available information about milk has been obtained on it. Cow's milk contains from 2 to 5 per cent of lipids, mostly fat, whereas the milk of smaller animals may contain much larger amounts. For example, the milk of the sow (Hughes and Hart, 1935) was found to contain 6.77 per cent of fat and that of the rabbit and rat still larger percentages.

Phospholipid and cholesterol are present in milk in small amounts, phospholipid of the order of 0.03-0.1 per cent, cholesterol 0.015 per cent.

Composition. The fat of cow's milk differs from the stored fat of the cow in several respects. It contains about 7 per cent of volatile fatty acids, mainly butyric and caproic, with small amounts of caprylic and capric acids and traces of many other acids. The non-volatile fatty acids consist of 30 to 40 per cent oleic acid and 60 to 70 per cent palmitic, with traces of lauric and arachidonic acids. There are present small amounts of phospholipid and of cholesterol. As noted, the first milk, or colostrom, is quite different in composition from the later milk. The fat has a higher melting point and it contains more cholesterol and lecithin.

An exhaustive study of the fat of cow's milk was made by Bosworth and Brown (1933). They found saturated even-numbered carbon acids from butyric to lignoceric and, in addition, some unusual unsaturated acids, such as decenoic, tetradecenoic, and tetracosenoic. They found no linoleic. Eckstein (1933) reported 0.17-0.25 per cent of linoleic and 0.07-0.17 per cent linolenic acid in butterfat; he found that the linolenic acid content could be increased by feeding linseed oil. Bosworth and Helz (1936; Helz and Bosworth, 1936) reported the presence of monohydroxy palmitic acid in butterfat; they also found cerotic acid. Hilditch and Longenecker (1938) found decenoic, dodecenoic, tetradecenoic, and hexadecenoic acids with double bonds in the 9-10 position, oleic, octadecadienoic, and arachidonic acids. Their hypothesis was that the lower fatty acids are produced by combined oxidation and reduction of oleic acid and that the minor unsaturated acids may be fragments which have escaped complete saturation.

The amount and nature of the phospholipid and the amount of cholesterol in milk have been the subject of considerable study. The amounts found are small and bear no relation either to the fat or to the other constituents, so that their function in milk is problematical. They seem to be in the nature of accidental additions coming from the blood, rather than purposeful constituents. The phospholipid content of cow's milk was given by Koch (1906) as lecithin 0.036 to 0.049 per cent and cephalin 0.072 to 0.086 per cent. Human milk had much the same content. Chapman (1928) gave figures for phospholipid in milk of 0.0447, cream 0.1981, skim milk 0.0165, buttermilk 0.1302 per cent. Wiese, Nair and Fleming (1932) reported a uniform phospholipid content in butter of between 18 and 20 mg. of phosphorus per 100 grams of fat. Lobstein and Flatter (1935) found phospholipids in cow's milk to be about 300 mg. per liter. The fat of casein was found by Stevenson and Bacharach (1937) to contain no phospholipid; otherwise it was the same as butterfat.

As the result of examination of the milk of the cow, goat, ass, and human being, Hess and Helman (1925) found no relation between fat and phospholipid content. There was more phospholipid in early than in late lactation. Cow's milk contained twice as much phospholipid as human milk. Total phosphorus of cow's milk was four times that of human and that of goat's milk was still higher. Diemair, Bleyer and Ott (1934) found that the methyl alcohol-soluble phospholipid was a mixture of lecithin and cephalin containing stearic, palmitic, and oleic acids. Kurtz, Jamieson and Holm (1934), examining the lecithin-cephalin fraction from sweet buttermilk powder, found no lower fatty acids and no palmitic acid. The high percentage of oleic acid indicated that much of the phospholipid contained only unsaturated acids. They found the following percentages of fatty acids: myristic 5.2, stearic 16.1, arachidic 1.8, oleic 70.6, and dicostetrenoic (?) 6.3 per cent.

Palmer and Wiese (1933) found that the protective coat around the fat particles in cream consisted of a mixture of protein and phospholipid. The protein appeared to be different (in nitrogen content) from the other milk protein, and the phospholipid contained both monoamino and diamino components. The percentage of protein and phospholipid was not constant (Rimpila and Palmer, 1935).

Meigs and Marsh (1913) found cholesterol in cow's milk to the amount of 0.021 per cent. Fox and Gardner (1924) found that the unsaponifiable substance of butterfat consisted of cholesterol, some low-melting oily substances of similar empirical composition to the sterols, and perhaps traces of sterol esters. Milk was found to be richer in cholesterol in early than in late lactation. Diet, if sufficient, had little effect. There was no relation between fat and cholesterol. Ansbacher and Supplee (1934) found that the cholesterol content of butter oil varied between 0.24 and 0.34 per cent, and that 18 per cent of the total milk cholesterol was associated with protein, mainly the lactalbumin.

In human milk, Denis and Minot (1918) found cholesterol values (colorimetric) of 0.091 to 0.038 per cent; in 100 grams of milk fat, 0.42 gram (digitonin precipitation). All the cholesterol was present as ester and the percentage decreased as lactation proceeded. The average fat content of these samples of human milk was 3.29 per cent. In comparison, eight samples of cow's milk gave an average of 3.65 per cent of fat and 0.13 per cent of cholesterol. Knauer (1928) reported values of 0.014 per cent of cholesterol in human milk in a secretion which may amount to 2 liters daily. Mühlbock (1934) found in mixed samples of human milk between the fourth and tenth days of lactation an average of 26 mg. of cholesterol per 100 cc., mostly free. The milk fat contained

an average of 1 per cent of total cholesterol. Cow's milk contained an average of 9.18 mg. per 100 cc.

Polonovski, Cuvelicr and Avenard (1932) obtained the following data on the fat of human milk from a composite sample collected during the first ten days of lactation:

	Per cent
Glycerol and unsaponifiable	7
Unsaturated acids as oleic	61
Saturated acids, chiefly myristic	31
Volatile acids insoluble in water	0.6
Caproic acid	0.35
Iodine number	54
Melting point (°C.)	30

Data on the changes in fat and other constituents of human milk both during the day and during the whole of lactation have been furnished by Nims and associates (1932). Characteristic daily trends in the amount of fat were found. In the course of lactation the fat at first diminished; then, later in lactation, it increased to the original high values. The fat content showed great differences in different individuals and at different times, varying from about 2 to 5.5 per cent.

Dhingra (1933) reported that the fatty acids of the milk fats of goats and sheep were similar in composition, but differed from those of cow or buffalo butter in having a higher content of the lower fatty acids. Riemenschneider and Ellis (1936) gave the following values for the component acids of goat's milk fat: decenoic 0.2, tetradecenoic 0.4, palmitoleic 2.1, oleic 31, arachidonic 0.7, butyric 2, caproic 2, caprylic 3, capric 8, lauric 3.5, myristic 10, palmitic 29, stearic 8, and cerotic 0.4 per cent.

In pigeon's crop milk, Read, Mendel and Vickery (1932) found: solids 14.3-18.6 per cent, fat 9.0-12.7 per cent; the iodine number of the cold alcohol extract was 106, of the hot extract 114, 115. When used as the sole nutriment, it contains adequate A and B vitamins.

The milk of monotremes differs from that of higher animals mainly in that there are no volatile fatty acids (Marston, 1926).

Origin of milk fat. The extent to which the fat of cow's milk depends on or is influenced by the fat of the food has been reviewed by Smith, Wells and Ewing (1916), and the general conclusion is that milk fat in cows is undoubtedly influenced by food fat, but that the effect is not immediate, requiring some days to become evident. These workers found that when cows were fed cottonseed oil, the properties of the butterfat differed from those of butterfat produced when the cows were not receiving cottonseed oil. The change in properties was greater, the larger the amount of oil. The changes produced by the oil ration did not occur with maximum intensity immediately, but showed a gradual increase up until

the seventh day of continued feeding, although certain constituents of cottonseed oil could be detected in the butterfat within twelve to thirty-six hours after feeding the oil. Cottonseed oil, when fed in small quantities, was not transferred in any considerable amounts directly to the milk fat, but some of the substances of which the oil is composed apparently were transferred. The constituents of the oil did not reach the milk fat in the same combinations or proportions in which they existed in cottonseed oil. In goats, Bowes (1915) found that the time for the fat of the food to appear in detectable amounts in the milk was less than twelve hours. Mendel and Daniels (1912) had previously reported that the fat-soluble dyes (Sudan III, Biebrich Scarlet, Oil Soluble Green, Oil Orange, and Butter Color) pass into the milk of animals accustomed to a fat diet (rats, cats) and into the egg fat of chickens, but do not appear in the milk of the cow.

Gage and Fish (1924) also present evidence to show that different species of animals vary greatly as to the effect of food fat on milk fat. In cats, dogs, and rats (carnivora and omnivora) stained food fat appeared promptly in the milk. In goats, the milk was only faintly stained, while in the cow it was not possible to demonstrate any staining of the milk fat after feeding stained fat. Gage and Fish conclude that the milk fat in the cow was derived mostly by a transformation of the carbohydrate and protein of the food, and that the fat of the food had only a minor influence. Gage's results with cows may be harmonized with those of Smith and others mentioned above by the probability that his experiments were of too short duration for food fat to show its effect on the milk fat. Possibly the fat is first deposited in the fat stores and only later used for milk fat. In carnivorous animals which are accustomed to much fat in their food, the transfer of fat from food to milk is prompt.

Maynard and McCay (1929) found that a ration low in fat lowered the milk yield in cows but had little effect on the fat percentage in the milk. There was a correspondingly lowered total fatty acid and cholesterol of the blood and a lowered iodine number of the milk fat. Further work by the same authors (McCay and Maynard, 1931) showed that during the period of low fat intake the phospholipid and total phosphorus of the plasma decreased, that of the erythrocytes being unaffected, which may be taken as further evidence of the relation of plasma phospholipid to milk fat. It is obvious from this work that cows secreting large amounts of milk fat cannot synthesize it fast enough, and that, if the outside source of fat is shut off, the fat output and with it the secretion of milk falls off, and coincidentally the level of the blood lipid falls. Buschmann (1930) found that addition of fat to the food did not

change the percentage of the fat in the milk, but did change the chemical nature of the milk fat. The addition of from 0.4 to 1.0 kilogram of oil per day per thousand kilograms body weight affected the milk fat as if 18 per cent of the added oil had gone directly into the milk. Maynard, McCay and Madsen (1936) found, in cows, that on changing from a more to a less unsaturated fat in the ration, and vice versa, that corresponding changes in the iodine number of the milk took place within 18 hours in half the animals after the change of ration, and in all cases within the next 24 hours, the maximum being reached in three or four days. Corresponding but less marked changes took place in the blood lipids.

Bender and Maynard (1932), in a study of lactating goats, found that the level of the blood lipids rose and fell parallel with the fat content of the diets, and that a low-fat or a coconut oil diet resulted in a secretion of milk fat which was much more saturated and lower in molecular weight than that on a high-fat ration. Leroy and associates (1931) found that there existed a high coefficient of correlation between the total fatty acids in the blood and the butterfat content of the milk. It was suggested that the determination of fatty acids in the blood would furnish a value by which the future capacity of young cattle to produce butter might be estimated. Its use in the breeding of cattle for high butterfat is also suggested.

A study was made by Maynard, Harrison and McCay (1931) of the changes in the blood lipids during the lactation cycle, with the following results. Determining the total fatty acids, phospholipid fatty acids, and cholesterol in the blood of four cows during the dry period and in the succeeding lactation, they found that after parturition there was a rapid and approximately parallel rise in all these constituents, succeeded by a gradual drop to the original levels as the next dry period approached. It was shown that the parallelism between the blood lipids, which was recognized to exist in fasting and non-lactating animals, was also exhibited as these values rose and fell during lactation, during which an intense fat metabolism was going on. A close metabolic relationship among these lipids was suggested. In experiments with animals held at a constant level of food and fat intake during the dry period and the earlier weeks of lactation, the same rise in the blood lipids occurred after the onset of milk secretion. This demonstrated that lactation had an influence upon the level of blood lipids independent of any effect which changes in the fat intake might exert. Maynard (1932) found that the level of the blood lipids in cows varied with the period of lactation and was higher in milking cows than in dry ones.

Ragsdale and Brody (1922) were able to show that, all other conditions being approximately the same, the lower the environmental tem-

perature, within the observed limits, the higher the percentage of fat in cow's milk. Ragsdale and Turner (1922), as the result of a study of some thousands of cows of different breeds, found a seasonal variation in fat content of the milk, it being lowest in summer and highest in winter. Low temperature was associated with high fat content.

Hilditch and Sleightholme (1930) found that fat added to the diet of cows was of minor importance in affecting the composition of butterfat as compared with changes in environmental conditions, *e.g.*, change from outdoor to indoor life, seasonal change in temperature, and character of the diet. The differences consisted mainly in the amount of unsaturated and lower saturated acids formed. Palmitic acid was the most constant (24 to 27 per cent) while stearic acid varied erratically.

The substance in the blood of cows from which the milk fat originates was thought by Meigs, Blatherwick and Cary (1919) to be phospholipid. They were able to show that the blood leaving the milk glands of milking cows contained less phospholipid and more inorganic phosphorus than the blood in the general circulation as shown by samples taken from the jugular vein. In favorable cases, they were able to show that the decrease in blood phospholipid was enough to account for all the fat of the milk, so that the blood phospholipid appeared to be the only source of milk fat. Later workers have not been able to repeat these results as regards phospholipid; thus the question of phospholipid as the mother substance of milk fat must be regarded as still open, although there is little question but that the fat in the food passes into the milk as noted.

Lintzel (1934), in studies on six goats and one cow, using arterial blood to compare with that from the udder vein (Meigs and associates used jugular vein blood), found no differences in phospholipid content and hence did not agree that blood phospholipid was the mother substance of milk fat. He thought that milk fat originated from the fat of the blood. Doulkin and Helman (1934) found a high negative correlation between the cholesterol content of the blood serum and the total milk production of the cow. There was a high positive correlation between the lecithin content of the serum and the butterfat content of the milk at the time of drawing the blood sample, which would seem to connect the serum phospholipid with the formation of milk fat as was found by Meigs, Blatherwick and Cary but by no other workers since. McCay and Maynard (1935) found that the total lipid in the mammary vein plasma in milking cows was consistently lower than in the jugular vein, while neither the lipid nor inorganic phosphorus values were different, thus excluding phospholipid from participation in milk fat formation and indicating that some other lipid, probably neutral fat, was concerned.

For the manufacture of milk fat, carbohydrate and fat can replace

each other in the food (Sheehy, 1921). Bürger and Rückert (1931) fed 150 grams of dextrose to a fasting goat and found a sixfold increase in milk fat at the next milking, a finding which indicated that the extra milk fat had its origin in the carbohydrate fed, but revealed nothing as to the place at which the fat was formed.

Petersen, Palmer and Eckles (1929) made studies on the time of secretion of the fat of milk. Their method was to compare the milk obtained from parts of the same gland before and after slaughter of the animal. As much milk was obtained from the gland *post mortem* as in life; therefore, the cow's udder contained all the liquid secreted at a milking. The fat content was much lower in that taken from the gland *post mortem*; therefore, fat was secreted at milking time. With milkings during life the fat rose continuously to the end of the milkings. In the gland after slaughter it rose to a maximum and then fell off toward the end of milking. They found also that the nature of the fat in the lactating gland differed widely from that of the non-lactating gland. The fat of the lactating gland was intermediate in composition between butterfat and body fat; the fat of the non-lactating gland was much like that in the surrounding adipose tissue. The iodine number of the fat of the lactating gland was less than that of the adipose tissue around the gland. From these facts they concluded that the fat in the mammary gland of lactating cows was concerned in the provision of the fat of milk. The lecithin content of the moist gland, which was 0.170 per cent by weight, they thought was not high enough to be a factor in the immediate fat production. Perfusion of the surviving gland, either with salt solution or with emulsion of corn oil, did not give any significant information. Taken together with the preceding, these results indicated that the mammary gland in the cow takes a definite part in the formation of milk fat, which would tend to explain the lack of relation between food fat and milk fat in herbivorous animals as compared with the direct connection noted in omnivorous and carnivorous animals as discussed above.

In summary, the evidence indicates that much of the milk fat originates from the neutral fat of the blood and this from the food fat either immediately, as in the carnivora and omnivora, or later after temporary storage and possible changes, as in the herbivora. The origin of the short-chain fatty acids is not explained. There may be considerable formation of fat from carbohydrate in the gland. Blood phospholipid as a source of milk fat is doubtful.

Eggs. The egg constitutes the only source of maintenance and growth of the young of most animals except mammals from the beginning of life until, in many cases, they can take care of themselves. It should therefore be the best material for investigation of chemical-embryological

problems. However, as in the case of milk, there is surprisingly little information available regarding the fatty constituents which make up the greater part of the total energy of the egg. The analytical material on the lipids of egg is fragmentary and, far from being useful in the study of embryological problems, it is not approximately complete in any instance. Since egg supplies food for the young animal from the beginning of development, whereas milk only carries on a development which is already well along at birth, the differences in milk and egg lipids are important. The chief known difference is that the egg has a large proportion of phospholipid and cholesterol, while milk has only traces of these substances. The embryo needs to be supplied with these substances in its early stages until it can supply its own by synthesis. In the mammals, these are supplied through the blood from the mother, as shown by Boyd and Wilson (1935). The fatty acids of both eggs and milk are largely those of the mother's food. Apparently the young animal can use these fatty acids from the beginning without adaptation unless the short-chain fatty acids in milk can be regarded as an adaptation. It seems likely that some other explanation should be sought since there are no such short-chain fragments in the egg.

Hen's egg contains about 10 per cent of lipid, practically all of which is contained in the yolk. The yolk is about one-third lipid, and of this about one-third is phospholipid and the remaining two-thirds fat and cholesterol. Cholesterol amounts to about 0.2 to 0.4 gram per egg, phospholipid about 2 grams.

The fatty acids of the lipids of egg, both fat and phospholipid, are greatly influenced by the fat of the food, so that an analysis of egg lipid does not mean much apart from the fat of the food of the adult. Liebermann (1888) found in egg oil: palmitic acid 38 per cent, oleic 40 per cent, and stearic 15 per cent. The yolk was laid down in rings, each ring meaning a time interval of 24 hours, and it took about four days for a laying hen to produce the greater part of the yolk. Gage and Fish (1924), after feeding stained fat to laying hens, were able to demonstrate visibly the deposition of food fat in egg yolks, and their results agree with those of Liebermann. Almquist, Lorenz and Burmester (1934) reported that cottonseed oil from cottonseed meal is deposited both in egg yolk and in the body stores. Cruickshank (1934) found, on a normal cereal ration with protein supplement, that the mixed fatty acids of egg fat contained 31 per cent solid acids, 47-51 per cent oleic, 15-19 per cent linoleic, and 2-3 per cent linolenic acids. Sueyoshi and Furukubo (1931) stated that the solid acids of egg were mainly isopalmitic; the unsaturated acids consisted of oleic (mainly), clupanodonic (C_{22}), and linoleic acids.

Hilditch, Jones and Rhead (1934) found only 30-35 per cent of

saturated acids in hen's depot fat, a definitely lower percentage than in the stores of mammals, which is hard to explain in view of the higher body temperature of the hen; 65 per cent of the total acids belonged to the C_{18} series and these were oleic 35-38 per cent, linoleic 20-22 per cent, and no acids more unsaturated. In addition, there were 7-8 per cent palmitoleic acid, traces of C_{20} and C_{22} unsaturated acids and of myristic acid, and stearic acid 5-7 per cent. Palmitic acid was always 25-30 molecules per cent as in all land-animal fats and never in marine-animal fats.

Mottram (1913) studied the degree of unsaturation of the fatty acids of hens' eggs. The mean value of the iodine number was fairly constant in all localities and breeds at between 80 and 90. There were some variations between individuals but great constancy in the individual. Incubation affected the degree of unsaturation very little, although during the first week there was an undoubted rise in degree of unsaturation, whether the eggs were fertile or infertile.

The lecithin of egg yolk was found by Levene and Rolf (1921) to contain stearic, palmitic, and oleic acids. In a later communication (1922) they reported iodine numbers of between 30 and 54 for the cadmium chloride compounds of lecithin. The unsaturated acids were oleic and a small quantity of linoleic and arachidonic.

Yokoyama (1934) prepared lecithin from hen's egg yolks, obtaining 747 grams from 6.5 kilograms of yolk. It consisted of about 75 per cent β-lecithin, the remainder being the α form. The α-lecithins yielded oleic, clupanodonic, and isopalmitic acids in the proportion of 72:2:26; the β-lecithins gave oleic and isopalmitic only. Nishimoto (1934) prepared cephalin from egg yolk, which yielded on hydrolysis palmitic, oleic, and arachidonic acids; the yield was 28.5 grams from 6.5 kilograms of egg yolk. Riemenschneider, Ellis and Titus (1938) obtained 323 grams of purified lecithin from 4.5 kilograms of fresh egg yolk. The fatty acids of this lecithin contained palmitic and stearic as the only saturated acids; oleic, linoleic, and clupanodonic as the unsaturated. In the neutral fat, they found a small amount of a lower saturated acid and a 9-10 hexadecenoic acid.

Masuda and Hori (1937) found about 20 per cent of phospholipids in the dried yolks of birds' eggs, and the ratio of phospholipid to lecithin was 1.45 for hen's egg, 1.3-1.37 for ducks, 1.77-1.93 for quail, and 1.22 for peacock. In fish eggs, the ratio was 1.11 for salmon, 1.11 for prawn, 1.6 for cod, 1.14 for carp, 2.39 for herring, 1.0 for shark, and 2.41 for yellowtail. During hatching of the eggs of the wild duck, the cephalin diminished gradually, while the lecithin remained constant through all the stages.

For cholesterol, Skarzynski (1936) gave the following values found in leghorn eggs: average weight of egg, 58 grams; cholesterol content of the whole egg 0.39-0.54 per cent, each egg containing then about 0.25 gram of cholesterol. Miyamori (1934) found in twenty-two kinds of birds that the cholesterol content of egg yolks varied little, averaging 1-2 per cent in all but two species.

King and Dolan (1933) prepared lysolecithin and lysocephalin from egg yolk by the action of rattlesnake venom on the phospholipids. These compounds had marked hemolytic properties and were hydrolyzed by extracts of intestinal mucosa faster than ordinary intact phospholipid.

Fukuda (1939) analyzed the eggs of a number of snakes and the following approximate average values are taken from his tables: water 75 per cent, fat 12 per cent with an iodine number of about 38, phospholipid 1 per cent, cholesterol 0.4 per cent, and total choline 0.1 per cent. The phospholipid was about half what it is in hen's egg; cholesterol was about the same.

The physiology of the egg-yolk lipids has been discussed in the chapter on metabolism of the lipids and will be only briefly reviewed here. The published results on the changes in cholesterol during incubation are conflicting. Kusui (1929) found a decrease up to about the fourteenth day, then an increase, indicating that the embryo can synthesize cholesterol. Dam (1928; 1929) found wide differences in different eggs. No certain answer can be given regarding changes in cholesterol.

Regarding the changes in phospholipid during incubation, Hanes (1912) found that during the first two weeks phospholipids are predominantly present in the liver; after this time cholesterol esters accumulate there; upon hatching, the ester disappears again (see also Entenman, Lorenz and Chaikoff, Chapter V, p. 340). Phosphorus used by the chick for bone formation is derived from the phospholipids. Jost and Sorg (1932) found that the ratio of total fat to phospholipid in the egg remained constant during development. Kugler (1936), studying the metabolism of development in the chick, found that the phospholipid metabolism reached its highest between the fifteenth and seventeenth days of incubation, which is the period of greatest yolk decrement and embryo increment of lipid phosphorus. Lecithin and cephalin paralleled each other, preserving a 3:1 ratio in both the yolk and embryo throughout the incubation period studied. The fifteenth to seventeenth day was the period of greatest transformation of lipid phosphorus into other forms, and was the time of greatest ossification of the embryonic bones.

Hayes (1938) made periodic analyses of total fat, sterol, and phospholipid during the first 40 hours of development of arbacia eggs. The sterol content remained unchanged, and phospholipid values varied

greatly, so that no conclusion could be drawn regarding this substance. The total fat of a million unfertilized eggs was 5.65 mg., of which 7.5 per cent was sterol and 38 per cent phospholipid.

Lymph and chyle

Human thoracic duct lymph from a fistula was examined by Reiser (1937). On a fat-free diet, the following were the approximate average values for the lipids: phospholipid 70 mg. per cent, cholesterol 30 mg. per cent of which 25 per cent was free, and neutral fat about 320 mg. per cent. The values for phospholipid and cholesterol were much lower than those for human blood plasma, the neutral fat higher. Rony, Mortimer and Ivy (1932) discovered that the lymph of fasting or phlorizinized dogs contained more fat and less sugar and cholesterol than the blood drawn at the same time. Twenty-four hours after a fat meal the lymph value for total fatty acids was 230 mg. per cent. After two to fourteen days' fast, the total fatty acids varied from 250 to 1030 mg. per 100 cc. of lymph and 157 to 371 mg. per 100 cc. of blood. The lymph fat was derived from the fat stores and not from the blood. In a later communication (1933), these workers found that the source of this extra fat was the intestine, since, when the intestine was removed, there was no extra fat in the lymph. They explained their findings to be the result of a flow of depot fat to the intestine, the purpose of which was twofold: (a) for the excretion of fat through the intestine, which is probably necessary to bring about the excretion of surplus cholesterol; or (b) for the making over of the stored fat by the intestine.

Transudates and exudates are of such variable composition that analytical data are not of much use. Macheboeuf and Fethke (1932) have reviewed the subject and given a series of analyses. There is no sharp dividing line between the two classes of fluid, but in general the transudates are lower in protein and lipids.

Cerebrospinal fluid

Levinson, Landenberger and Howell (1921) could find no appreciable amount of cholesterol in normal cerebrospinal fluid, whereas fluids from hemorrhage of the brain and brain abscess were high in cholesterol. Lasch (1924) similarly could find no cholesterol in forty samples examined. Fabris (1921), in normal children, found 0.01 per cent of cholesterol in the cerebrospinal fluid. In hydrocephalus, cholesterol was absent; in tubercular meningitis it was increased. Cantieri (1916), however, was able to find no regularity in cholesterol content in various pathological conditions. Weston (1915) found cholesterol in the cerebrospinal fluid of persons who had suffered from mental diseases.

Bile

Bile is the normal external secretion of the liver and is delivered into the intestine at the duodenum. In most animals, there is a gall bladder into which the more or less continuously secreted bile is collected and concentrated, to be delivered later on suitable stimulus. The most common stimulus is the presence of fat in the intestine, and the main function of bile is in the digestion and absorption of fat. Bile normally contains small amounts of lipids: cholesterol, phospholipid, fat, soaps, and free fatty acids, of which the quantity varies with the nature of the food. The bile serves as one path of excretion of free cholesterol but probably not a very important one, the intestine and especially the large intestine being apparently the main path of excretion of this substance. The presence of cholesterol in the bile and especially in the concentrated bile of the gall bladder often results in deposition in crystalline form as gallstones, sometimes consisting of nearly pure cholesterol, but generally containing a variety of impurities such as bile pigments, calcium salts, and other bile constituents. The conditions under which gallstones are formed has been the subject of much speculation which has not resulted in any satisfactory explanation of gallstone formation.

Gardner (1924) stated that cholesterol was eliminated by the liver through the bile and reabsorbed from the intestine. Whether any was excreted by the intestine, and in what form, depended on the amount in the food. In carnivora, even on low intake, from 0.03 to 0.05 gram was excreted per day. In man there was a negative balance of 0.3 gram per day, so that there was probably some synthesis. In man, cholesterol was largely reduced to coprosterol and β-cholesterol, although some escaped change. Cholic acid is closely related to coprosterol and Windaus thought that bile acid originated in cholesterol. No evidence of such an origin has been produced, however. Gardner and Gainsborough (1930) emphasized the relation of the bile acids to cholesterol and other sterols, the cholagogue effect of the cholic acids on hepatic bile, and the relation of cholic acid to the absorption of fat and cholesterol.

McMaster (1924) found that when a diet rich in cholesterol was given to a dog, the amount of cholesterol in the bile increased greatly. Not only the total amount but the concentration per cubic centimeter was greater in almost every instance. An increase in the total food intake by the addition to the ordinary ration of a bone ash diet containing only a slight amount of additional cholesterol (200 mg.) produced a similar but lesser increase. In the fasting dog, the cholesterol yield was greatly cut down. The increase in cholesterol, after a diet rich in this substance, did not depend upon the cholagogue action of the food, though it was true that the concentration in the bile usually increased with the bile

volume. In spite of the fact that fasting cut down the cholesterol of the bile, the concentration of the substance per cubic centimeter was greatly increased. On an ordinary diet, the yield in the bile fluctuated abruptly and considerably from day to day. In general, the rule held that an animal eating heavily put out not only much more bile but much more cholesterol. The relation between the bile quantity and cholesterol yield was anything but a fixed one, however. The cholesterol yield of the bile did not parallel that of bilirubin. The pigment output from day to day remained relatively constant as compared with that of cholesterol. Actually, however, the amount of cholesterol put out in the bile was only a small fraction of the amount fed. Thus, on feeding of eggs and brains containing probably about 5-6 grams of cholesterol, the daily output in the bile was of the order of 70 mg., or less than 2 per cent of the intake.

In human bile, obtained by drainage after a gallstone operation, Nathan (1920) found constant values of 0.045 to 0.055 per cent of cholesterol after the third day. Pribram (1923) found that after childbirth there was an elimination of cholesterol from the blood of the mother and an increase in the bile with a thickening of the bile both in the liver and in the gall bladder. In pregnancy there was a corresponding decrease of cholesterol in the bile and an increase in the blood. Wichert and Russjajewa-Oparina (1925) found that the cholesterol content of human fistula bile varied from 0.02 to 0.7 per cent, bladder bile from 0.06 to 1.07 per cent. Normally, there was a relation between the cholesterol content of the blood and bile. Eggs, brain, and butter were found by Salomon (1926) to increase bile cholesterol. The following data were given in mg. of cholesterol per 100 cc. of bile:

Protein diet	13 to 21
Protein plus 4 eggs	25 to 35
Protein plus 200 grams butter	38 to 76

In this case also, the output of extra cholesterol was only a small percentage of the cholesterol of the eggs consumed. Salomon and Silva (1926) examined the duodenal fluid of a human subject and found that ten days on a cholesterol-free diet (bananas) reduced the cholesterol content of the fluid to about 10-12 mg. per cent. On a diet of eggs and calf brain, the cholesterol content rose to about 30 mg. per cent. When one diet was changed to the other, the cholesterol excretion varied with the intake but was seen to lag considerably.

Le Count and Long (1914) studied the fat content of liver and bile in man. In normal livers (21.6 to 28.8 per cent fat on basis of dry weight), the bile fat varied from 0.5 to 1.1 per cent; in abnormal livers (44.1 to 58.7 per cent fat), it varied from 1.5 to 2.4 per cent.

McClure, Huntsinger and Fernald (1934) studied the composition of

human bile collected with a duodenal tube after the flow had been stimulated by the introduction of cottonseed oil, beef peptone, or glucose. Cottonseed oil increased the soaps of the bile severalfold, with lesser increases of fat and free fatty acid. The organic phosphorus was also greatly increased. Bromine number studies of the blood and bile fatty acids indicated that the bile lipids are the result of a secretion rather than of filtration from the blood. Glucose had little effect on the lipid composition of the bile, and peptone had an effect intermediate between that of glucose and cottonseed oil.

Riegel, Ravdin and Rose (1937) examined the cholesterol content of bile under various conditions. They found that there was no correlation between fluid intake and cholesterol concentration or between the amount of bile drained externally and the cholesterol content. In two patients, no relationship was observed between the total 24-hour volume and the cholesterol concentration or between the amount of bile drained externally and the cholesterol content. Cholesterol concentration and bile salt concentration, in general, ran parallel with each other. In patients with badly damaged livers, the cholesterol concentration in hepatic bile was low, but it was high in those with slightly damaged livers. These investigators (1936) found, in agreement with earlier workers (Thannhauser, 1923; Wright, 1934), only free cholesterol in the bile from most individuals, but in one patient, the bladder bile contained 29 per cent of the cholesterol in ester form 6 days after operation. The blood plasma of this individual gave cholesterol values of 268 to 316 mg. per cent with 67 per cent as ester.

In hippopotamus bile, Gardner (1924) found a total cholesterol content of 22.4 mg. per 100 cc. of which less than 3 mg. was esterified.

Excretions

Feces

The feces contain about one-third of their dry weight of fatty material, which consists of fatty acids, either as such or as compounds (soaps) with the bases commonly present, sodium, calcium, and magnesium. Normally, there is not more than a trace of neutral fat or phospholipid. In addition to the fatty acids, there are always found various sterols, of which cholesterol and its reduced forms, dihydrocholesterol and coprosterol, are the commonest. These substances are now definitely recognized as excretions and not as unabsorbed food material.

The fecal sterols are made up of unabsorbable sterols such as those of plants and the excess cholesterol of the body either ingested or synthesized. The fatty acids are more closely related to those found in the

blood than to the food fatty acids, although the food fat has some influence on the nature and amount of the fecal fatty acids. The meaning of the fatty acids in the feces is not known. According to present information, they are the common fatty acids of the organism: palmitic, stearic, oleic, and a small amount of arachidonic, together with the commoner volatile fatty acids, butyric and caproic; the latter may have their origin in carbohydrate or protein as well as fat.

Secretion of fatty material into the intestine is well known and takes place even in fasting. The secreted material is largely reabsorbed and appears in the chyle, which may be one of the mechanisms for bringing into use the fat of the stores as indicated by the work of Rony, Mortimer and Ivy (1932). The need of fatty acids as a vehicle for sterol excretion has been suggested and the fatty acids so used may not be reabsorbed. Other suggested sources of fecal fatty acids are the cellular material desquamated from the intestine and also the bacterial debris which makes up a considerable portion of the fecal mass.

The sterols are excreted partly through the bile, but probably for the most part directly into the intestine. The cholesterol undergoes considerable reduction to dihydrocholesterol and coprosterol, possibly as a means of avoiding reabsorption. An analogy to the skin secretion should probably be kept in mind, since there is a considerable loss of fatty material through the skin, mainly in the form of cholesterol esters of the fatty acids; and it is quite possible that the excretion into the intestine may be of a similar nature.

The presence of fatty substances in the feces has been known for a long time, the first reference to it being that of Home (1813). Müller (1884) concluded that the material must be an excretion since it occurs in hunger. He found in dogs that the hunger feces contained about 20 to 47 per cent of fat which consisted mainly of free fatty acids with smaller amounts of cholesterol and soaps. The hunger feces of a professional faster (Müller, 1893) contained 28.42 per cent of lipid on a dry weight basis, of which free fatty acid was 41.5 per cent, soaps 11.5 per cent, and the rest neutral fat and cholesterol. In spite of these observations, the lipid material found in studies on fat utilization has mostly been regarded as unabsorbed fat.

In general, the fatty acids of the feces have been found to have a higher melting point than those of the food, which, having in mind the idea that they represented unabsorbed food fat, has been interpreted to mean that there was a selection of the softer fat during absorption. Thus, Müller (1887) found that when dogs were fed lard (m.p. 43°C.) the melting point of the feces fatty acids was 50.5°C.; when they were fed mutton fat (m.p. 52°C.), the value was 56.0°C. In a man on a milk diet,

the feces fatty acids melted at 50 to 51.5°C., while the milk fat had a melting point of 43°C. Munk (1890) found in humans fed lard (m.p. 35-37°C.) that the melting point of the feces fatty acids was 43-46°C.; in those fed mutton fat (m.p. 50-53°C.) the value was 51-54°C. Hecht (1905) reported that feces fat, in general, was 4 to 8 degrees higher in melting point than food fat.

In attempting to determine whether an excretion of fatty material into the intestine actually took place, investigators have from time to time examined the contents of intestinal loops and collected the liquid passing from fistulas of various kinds (Gumilewski, 1886; Röhmann, 1887; Hermann, 1890; Voit, 1892; Kobert and Koch, 1894). They found an undoubted excretion of fatty material which they have variously attributed to cellular material and to true excretion. Ehrenthal (1891), as the result of examination of material supplied by Hermann (1890) and that collected by himself, concluded that the fatty material was of epithelial origin. Voit (1892) attributed the material to excretion. He found that from 23 to 36 per cent of the material collected was fatty in nature, consisting of from 1 to 10 per cent neutral fat, from 13 to 30 per cent fatty acids, and from 3 to 8 per cent soaps. Kobert and Koch (1894), in a human case with a fistula of the large intestine, found that 1 gram of dried material contained 6.8 to 9.3 per cent of lipid, of which 90 per cent was fatty acid, 9 per cent fat, and 1 per cent soap.

Holmes and Kerr (1923) found that the fat of the diet had but little influence on the fat of the feces. In experiments on humans with various easily digested fats, ether extracts of the feces were practically the same for each, *i.e.*, they had iodine numbers from 28 to 34, saponification values of 108 to 159; values were widely different from those of the fat fed. They found no appreciable amount of soap. The ether-soluble unsaponifiable matter consisted largely of coprosterol, melting point 94.5°C., and also a small amount of unidentified sterol.

Work by Hill and Bloor (1922) and Sperry and Bloor (1924) has shown that the fecal lipids are to a large extent independent of the fat of the food, although noticeably influenced by it. There was a decided, although not large, increase in total lipid excretion upon feeding diets containing fat when contrasted with the excretion on a fat-free diet. Sperry (1926) concluded from this that it was probable that there was a basal excretion of lipid material most nearly represented by that obtained on a lipid-free diet, and that in the case of fatty diets this basal excretion was augmented by a small amount of lipid which had either escaped absorption or had been absorbed and re-excreted. Sperry found in dogs of from 5 to 13 kilograms in weight on a lipid-free diet that there was an excretion of from 1.5 to 2 grams of non-volatile lipid per week,

made up of 35 to 40 per cent unsaponifiable material and 55 to 60 per cent fatty acids which contained 30 per cent solid and 60 per cent liquid acids. The solid acids consisted of palmitic and stearic, with stearic in slightly larger amounts; the liquid acids consisted mainly of oleic with a small amount of a four-bond acid, probably arachidonic. This excretion on a fat-free diet continued at about the same level for five weeks, although there was a slight falling off toward the end. The composition was remarkably uniform throughout. The volatile acids were found to be approximately 65 per cent acetic, 23 per cent butyric, and 12 per cent caproic. Cecchini (1923) found that acetic and butyric were the main volatile fatty acids of the feces. They were derived mainly from carbohydrates, but not necessarily so since they might have come from amino acids. They were generally in inverse relation to the indican content.

Angevine (1929) examined the material collected from Thiry-Vella fistulas at different levels in the intestinal tract and found in two dogs, one with a high jejunal fistula and the other with a fistula of the lower ileum, each about 12 inches in length, that the excretion amounted to about 2.2 mg. per day for the high fistula and 2.35 mg. for the low one, and that the amount excreted was independent of the diet.

Thus it has been shown that the lipids of the feces represent excreted material to a great extent, rather than unabsorbed food fat. It has also been possible to evaluate other sources of fecal lipids. In an attempt to discover the effect of bile on lipid excretion, Sperry (1927) excluded the bile from the intestine by means of a fistula, and found that the lipid excretion on strictly lipid-free diets was 1.5 to 4.5 times greater than the average normal excretion. It did not diminish from week to week, and the composition was much the same as in the normal animals. This work eliminates the bile as a source of fecal lipids.

Furthermore, by the use of a modified Strasburger procedure, Sperry separated the bacteria from the other particulate matter of feces and found that the composition of the bacterial lipids was much the same as that of the particulate non-bacterial material, and that the lipids of both may represent adsorbed material. Sperry and Angevine (1932) studied the lipid excretion of the small and of the large intestine separately, and came to the conclusion that the excretion could not be of bacterial origin because of its high sterol content. The amounts secreted were much higher than had been found in the feces, indicating some degree of reabsorption. Reabsorption of cholesterol was definitely shown by Schoenheimer and Hrdina (1932) by means of a sterile intestinal cyst. Fatty acids and cholesterol were readily absorbed; dihydrocholesterol was not.

Sperry (1932), by analyses of the mucosa of the intestine, was able

to eliminate the possibility that the fecal lipids originated in desquamated mucosal epithelium. He found, as Bürger and Oeter (1929b) had previously found, that the amounts of lipid in the mucosa were too small to account for the excretion, even if the whole mucosa was removed every week. The average endogenous lipid excretion he found to be about 219 mg. per kilogram body weight per week for normal dogs and 615 mg. for bile-fistula dogs. The figures of Bürger and Oeter were: 0.646 gram of cholesterol in 100 grams of dry large intestine and 1.815 grams in 100 grams of dry mucous membrane from the same. The values are notably small, only 78 mg. for a whole large intestine, which is less than is given off in one day. Desquamation cannot therefore account for the cholesterol in the feces.

Sperry and Angevine (1932) were able to show that the sterol part of the excretion took place to a considerable extent in the large bowel. Beumer and Hepner (1928) reported that cholesterol introduced intravenously was not excreted by way of the bile but directly into the intestinal tract. They (1929) also reported finding a much higher cholesterol content in the colon than in the ileum. In a bile-fistula dog after a lipid-free meal, there was found an even more marked difference between the two portions of the intestine. The dried content of the ileum contained 0.21 per cent of cholesterol as compared with 1.21 per cent in the colon. Bürger and Oeter (1929a) found a greater cholesterol content in the sigmoid of cadavers than in sections of the small intestine.

The modern work on lipid excretion by the feces thus eliminates the bile as an important path of excretion of the lipids and makes doubtful intestinal bacteria and desquamated epithelium as important sources of fecal lipids, at the same time leaving no doubt that there is a considerable true excretion of lipid material. The excretion would undoubtedly be much greater if a considerable portion, perhaps most, of what had been excreted in the upper part of the intestine were not reabsorbed lower down.

Of interest is the work of Hill and Koehler (1932), who found that on a low-fat diet, epinephrine in subglycosuric amounts usually caused a definite (100 per cent) rise in fecal lipid excretion on the day following. The significance of these results with respect to the mechanism of lipid excretion has not yet been explained.

Schoenheimer and von Behring (1930) examined the sterile secretion of the large intestines in dogs and found 80 per cent of the collected lipids to be unsaponifiable matter. Gardner and Gainsborough (1930) agree with Schoenheimer (1929) that plant sterols cannot be changed into cholesterol in the animal body. Carnivora and omnivora have a good excretory mechanism for cholesterol, excreting it largely as coprosterol

and β-cholesterol, while the herbivora do not excrete cholesterol readily, so that it accumulates in the blood and tends to deposit in the tissues.

The sterols of human feces were found by Bürger and Winterseel (1929) to consist ordinarily of about 50 per cent coprosterol and the rest cholesterol; 10 to 30 per cent of the sterols was esterified. Even a large dose of cholesterol (5 grams in 100 cc. of oil) did not change the ratio greatly, partly because it is not promptly excreted; even in 6 days only a relatively small portion is excreted. On a one-sided diet of milk and eggs, most of the excreted sterol was cholesterol. Work by Bischoff (1930) indicates that the necessary reduction of cholesterol to coprosterol via allocholesterol is brought about by some agent in the feces. The bromine-binding power of fecal cholesterol diminishes greatly when the material is kept at 38°C. Allocholesterol is difficult to reduce outside of the intestine.

Studying calves during the first seven days of life, Howe (1921) found that the fat content of feces reached the high point on approximately the third day after birth. This high fat was accompanied by a relatively large percentage of soap. The dry residue formed about 38, 38, 34, 31, 37, 34, and 33 per cent of the moist feces on consecutive days starting from the first day; the percentage of the total fat was correspondingly 14, 14, 22, 11, 13, 11, and 13 per cent of the total solids; the percentage of soap was 1, 2, 4, 0.4, 0.5, 0.6, and 0.7 per cent on these days. The soap was chiefly calcium stearate.

In the stools of infants, Wacker and Beck (1921) determined that the free fatty acids consisted exclusively of stearic and palmitic acids; volatile fatty acids or acids of lower molecular weight were not found. In the fatty stools of infants, Bosworth, Bowditch and Giblin (1918) noted that the fatty material often consisted almost entirely of calcium palmitate. Holt, Courtney and Fales (1919) have recorded a number of studies on fat excretion in infants and young children, some data from which are given in Table 42.

Table 42. Average Normal Values for Fat Excretion in Infants and Young Children (Holt, Courtney and Fales, 1919).

	Total Fat (% of dry substance)	Soaps (% of total fat)	Neutral Fat (% of total fat)	Fat Utilization (%)
Breast fed infants	34.5	57.8	15.9	90.3–99.2
Infants on cow's milk	36.2	72.8	9.4	91.3
Children 1 to 10 years on milk or milk with bread and cereal	30.7	60.9	14.0	90.6
On mixed diet	18.0	45.1	27.5	93.9

Diarrhoea resulted in losses up to 42 per cent of the intake, of which 60 per cent might be neutral fat.

In the condition known as steatorrhea, the main abnormality is the wastage of food fat by the intestine. Garrod and Hurtley (1913) examined a patient with steatorrhea who was otherwise normal. There were no other signs of pancreatic disease; the bile was normal, and on a low-fat diet the stools were almost normal in appearance. When the diet was rich in fat, splitting was imperfect, but on a diet of 90 grams of fat per day, it was within normal limits. Improved splitting was not followed by improved absorption, so that the fault appeared to be in the absorption. Thaysen (1926) found steatorrhea in two conditions, diabetes and sprue. In sprue, the loss of fat amounted to 75 per cent and was not accompanied by nitrogen loss, whereas in diabetes there was nitrogen loss. In alcoholic steatorrhea, there was normal nitrogen excretion, but in the pancreatic form, there was increased nitrogen excretion. Thomas and Schlutz (1938), in a case of pancreatic steatorrhea, found no free fatty acids in the stool. The stool analysis showed: water 71 per cent, fat (dry weight) 42 per cent, free fatty acids none, neutral fat 56 per cent, unsaponifiable matter 30 per cent, and fatty acids as soaps 14 per cent.

Urine

Mörner (1895) was the first to record the finding of high molecular fatty acids in the urine. Kakiuchi (1911) also reported fatty acids. Carter (1916) found in a case of chyluria, which was probably the result of filariasis, although no filaria could be found in the blood, that the urine contained blood and fat, the amount of fat depending on the fat of diet. On a fat-poor diet (14 grams per day) urine fat was 0.35 per cent and the total output 3.4 grams. On the house diet, urine fat rose to 1-1.4 per cent. On a diet containing 80 grams of fat, urine fat was 0.9 per cent, a total of 6.5 grams. There was no difference between day and night so that posture was not a factor. Sano (1920) found in chyluria large amounts of both cholesterol and lecithin in urine, with the cholesterol mostly esterified.

Bauman and Hansmann (1920b) found a daily total lipid excretion in the urine of 8.5 mg. which could be increased fourfold by fat feeding. They found that in parenchymatous nephritis as high as 73 mg. could be obtained, indicating increased kidney permeability for lipid. They (1920a) collected clinical, pathological, and chemical data on a case of lipuria associated with nephritis which terminated in uremia and found that the lipuria was influenced by the amount of fat in the diet. The absence of coagulated protein, the scarcity or absence of cells in the urine, and the apparent absence of a fistulous communication between the urinary passages and the lymphatics at autopsy indicated that the

lipuria was due to an altered permeability of the renal cells. The available evidence makes it probable that there are at least two types of lipuria, the one associated with a fistulous communication, and the other entirely due to an abnormal condition of the kidney cells. Knauer (1928) reported an active lipid excretion in nephrosis. Page (1936) found that in nephritic patients, lipid nitrogen and phosphorus are present only in traces and do not vary with the protein output.

Faerber (1926) found that the urine of healthy children was fat-free in contrast to that of adults. Dogs sometimes showed a physiological excretion of fat.

Gardner and Gainsborough (1925) reviewed the literature on the occurrence of cholesterol in urine and reported that normal urine contained measurable amounts of sterol precipitable by digitonin, partly free, partly as ester, and partly in a form hydrolyzable by acids but not by alkalis. Total cholesterol in normal urine was found to the extent of 1.7 to 4.2 mg. per day. In the urine of parenchymatous nephritis it might reach 42 mg. In albuminous urines the cholesterol and its esters were combined with the protein much as they were in blood. They found the highest urine cholesterol content in hypercholesterolemia.

Bloch and Sobotka (1938) found from 0.2 to 0.5 mg. per liter in normal urine, and 3.5 to 7 mg. per liter in the urine of cancer. High values were found in kidney disease and pregnancy. Butenandt and Dannenbaum (1937) found 0.75-1 mg. of cholesterol daily in normal human urine.

Wichert, Pospeloff and Jakowlewa (1929) could find increased urinary cholesterol only in nephrosis. Grunke (1922) found only traces in normal urine (1 mg. daily); nine cases of icterus averaged 10 mg., and one diabetic 13 mg. Gaál (1930) found cholesterol in the urine in notable amounts only in nephrosis; in other kidney diseases only in traces. The amount in the urine varied with the blood cholesterol level but seemed to be independent of feeding.

The sex hormone from pregnancy urine, pregnandiol (Butenandt, Hildebrandt and Brücher, 1931), belongs to the group of sterol-like substances which are excreted.

Skin

Birds and mammals excrete much lipid through and onto the skin. In humans, Hueck (1925) found a widely variable daily excretion of 0.1 to 0.2 gram cholesterol.

Barbour, Dawson and Neuwirth (1925) found that lipids, together with water and salts, increased in the blood preliminary to sweating. As the sweating progressed the salts remained high while the lipids fell.

Samples of sweat, according to Pemberton, Cajori and Crouter (1929) contained lipids in mg. per cent as follows: 103, 44, 66, 216, 184, 25, 20, 83, 26, 72, and 134.

Bibliography

Almquist, H. J., Lorenz, F. W. and Burmester, B. R., *J. Biol. Chem*, **106**: 365 (1934). p. 360
Angevine, R. W., *J. Biol. Chem.*, **82**: 559 (1929). p. 369
See also Sperry, W. M.
Ansbacher, S. and Supplee, G. C., *J. Biol. Chem.*, **105**: 391 (1934). p. 354
Avenard, R., *see* Polonovski, M.
Bacharach, A. L., *see* Stevenson, S. G.
Barbour, H. G., Dawson, M. H. and Neuwirth, I., *Am. J. Physiol.*, **74**: 204 (1925). p. 373
Barjot, G, *see* Leroy, A.
Bauman, L. and Hansmann, G. H., *Proc. Soc. Exptl. Biol. Med*, **17**: 115 (1920a). p. 372
J Am. Med. Assoc., **74**: 1375 (1920b). p. 372
Beck, K. F., *see* Wacker, L.
Behring, H. v., *see* Schoenheimer, R.
Bender, R C. and Maynard, L A., *J. Dairy Sci*, **15**: 242 (1932); *C. A.*, **26**: 4088 (1932). p. 357
Beumer, H. and Hepner, F, *Klin. Wochschr*, **7**: 1470 (1928); *Z. ges. exptl. Med.*, **64**: 787 (1929). p 370
Bhattacharya, R. and Hilditch, T P., *Proc. Roy. Soc. London*, **A129**: 468 (1930). p 351
Bickenbach, W. and Rupp, H., *Z. Geburtsh. Gynäkol*, **100**: 1 (1933). p 351
Bischoff, G., *Biochem Z.*, **222**: 211 (1930). p. 371
Blatherwick, N. R., *see* Meigs, E. B.
Bleyer, B, *see* Diemair, W.
Bloch, E. and Sobotka, H, *J. Biol. Chem.*, **124**: 567 (1938). p. 373
Bloor, W. R., *see* Hill, E, Sperry, W. M
Bosworth, A. W., *see* Helz, G. E.
Bosworth, A. W. and Brown, J. B., *J. Biol. Chem.*, **103**: 115 (1933). p. 353
Bosworth, A. W. and Helz, G. E., *J. Biol. Chem.*, **112**: 489 (1936). p. 353
Bosworth, A. W., Bowditch, H. and Giblin, L. A., *Am. J. Diseases Children*, **15**: 397 (1918). p. 371
Bowditch, H., *see* Bosworth, A. W.
Bowes, O C., *J Biol. Chem.*, **22**: 11 (1915). p. 356
Boyd, E. M., *Biochem. J.*, **29**: 985 (1935). p. 352
Boyd, E. M. and Wilson, K. M., *J. Clin. Investigation*, **14**: 7 (1935). p 352, 360
Brody, S., *see* Ragsdale, A. C.
Brown, J. B., *see* Bosworth, A. W.
Brown, M., *see* Nims, B.
Brücher, H., *see* Butenandt, A.
Bürger, M. and Oeter, H. D., *Z. physiol. Chem.*, **182**: 141 (1929a); **184**: 257 (1929b). p. 370
Bürger, M. and Rückert, W., *Z. physiol. Chem.*, **196**: 169 (1931). p 359
Bürger, M. and Winterseel, W., *Z. physiol. Chem.*, **181**: 255 (1929). p 371
Burmester, B R, *see* Almquist, H. J.
Buschmann, A., *Tierernähr.*, **1**: 129 (1930); *C. A.*, **25**: 1558 (1931). p. 356
Butenandt, A. and Dannenbaum, H., *Z. physiol. Chem.*, **248**: 151 (1937). p 373
Butenandt, A., Hildebrandt, F. and Brücher, H., *Ber. deut. chem Ges.*, **64B**: 2529 (1931). p. 373
Cajori, F. A., *see* Pemberton, R.
Cantieri, C., *Riforma Med.*, **32**: 1009 (1916); *C. A.*, **11**: 561 (1917). p 363
Carter, D. W., *Arch. Internal Med.*, **18**: 451 (1916). p. 372
Cary, C. A., *see* Meigs, E. B.
Cecchini, A., *Arch. patol. clin. med.*, **2**: 361 (1923). p. 369
Chapman, O. W., *J. Dairy Sci.*, **11**: 429 (1928). p. 353
Courtney, A. M., *see* Holt, L E.
Crouter, C. Y., *see* Pemberton, R.
Cruickshank, E. M., *Biochem. J.*, **28**: 965 (1934). p. 360
Cuvelier, L., *see* Polonovski, M.
Dam, H., *Biochem. Z.*, **194**: 188 (1928); **215**: 475 (1929). p. 362
Daniels, A. L., *see* Mendel, L. B.
Dannenbaum, H., *see* Butenandt, A.
Dawson, M. H., *see* Barbour, H. G.
Denis, W. and Minot, A. S., *J. Biol. Chem.*, **36**: 59 (1918). p. 354
Dhingra, D. R., *Biochem. J.*, **27**: 851 (1933). p. 355
Diemair, W., Bleyer, B. and Ott, M., *Biochem. Z.*, 119 (1934). p. 354
Dolan, M., *see* King, E. J.
Doulkin, A. and Helman, S., *Lait*, **14**: 797 (1934); *C. A.*, **29**: 835 (1935). p. 358
Eckles, C. H., *see* Petersen, W. E.
Eckstein, H. C., *J. Biol. Chem.*, **103**: 135 (1933). p. 353
Ehrenthal, W., *Arch. ges. Physiol. (Pflügers)*, **48**: 74 (1891). p. 368
Ellis, N. R., *see* Riemenschneider, R. W.
Ewing, P. V., *see* Smith, F. H.
Fabris, S., *Pediatria*, **29**: 1057 (1921). p. 363
Faerber, E., *Z. physiol. Chem.*, **154**: 302 (1926). p. 373
Fales, H. L., *see* Holt, L. E.
Fernald, A. T., *see* McClure, C. W.
Fethke, N., *see* Macheboeuf, M.
Fish, P. A., *see* Gage, S. H.
Flatter, M., *see* Lobstein, J. E.
Fleming, R. S., *see* Wiese, H. F.
Fox, F. W. and Gardner, J. A., *Biochem. J.*, **18**: 127 (1924). p. 354
Fukuda, S., *J. Biochem. (Japan)*, **30**: 125 (1939). p. 362

Furukubo, T., *see* Sueyoshi, Y.
Gaál, A. M., *Z. ges. exptl. Med.*, **71**: 690 (1930). p. 373
Gage, S. H. and Fish, P. A., *Am. J. Anat.*, **34**: 1 (1924). p. 356, 360
Gainsborough, H., *see* Gardner, J. A.
Gardner, J. A., *Biochem. J.*, **18**: 777 (1924). p. 364, 366
See also Fox, F. W.
Gardner, J. A. and Gainsborough, H., *Biochem. J.*, **19**: 667 (1925). p. 373
Quart J. Med., **23**: 465 (1930). p. 364, 370
Garrod, A. E. and Hurtley, W. H., *Quart. J. Med.*, **6**: 242 (1913). p. 372
Giblin, L. A., *see* Bosworth, A. W.
Grunke, W., *Biochem Z.*, **132**: 543 (1922). p. 373
Gumilewski, —, *Arch. ges Physiol.* (*Pflügers*), **39**: 556 (1886). p 368
Hanes, F. M., *J Exptl. Med.*, **16**: 512 (1912). p. 362
Hansmann, G. H., *see* Bauman, L.
Harrison, E. S, *see* Maynard, L. A.
Hart, H. G., *see* Hughes, E. H.
Hayes, F. R, *Biol Bull*, **74**: 267 (1938). p 362
Hecht, A. F., *Jahrb. Kinderheilk.*, **62**: 613 (1905). p 368
Helman, F D, *see* Hess, A. F.
Helman, S., *see* Doulkin, A.
Helz, G E., *see* Bosworth, A. W.
Helz, G E. and Bosworth, A. W., *J. Biol. Chem*, **116**: 203 (1936). p. 353
Hepner, F, *see* Beumer, H.
Hermann, L, *Arch ges. Physiol.* (*Pflügers*), **46**· 93 (1890). p. 368
Hess, A. F and Helman, F D., *J Biol. Chem*, **64**: 781 (1925). p. 354
Hildebrandt, F., *see* Butenandt, A.
Hilditch, T. P, *see* Bhattacharya, R.
Hilditch, T. P. and Longenecker, H. E, *J. Biol. Chem.*, **122**: 497 (1938). p 353
Hilditch, T. P and Sleightholme, J. J., *Biochem J*, **24**: 1098 (1930). p. 358
Hilditch, T P, Jones, E. C and Rhead, A. J., *Biochem J.*, **28**. 786 (1934). p 360
Hill, E and Bloor, W. R, *J. Biol. Chem*, **53**. 171 (1922) p. 368
Hill, E and Koehler, A E., *J Biol Chem*, **98**: 185 (1932). p 370
Holm, G. E., *see* Kurtz, F. E.
Holmes, A D. and Kerr, R. H., *J. Biol. Chem.*, **58**· 377 (1923). p. 368
Holt, L. E, Courtney, A. M. and Fales, H. L., *Am. J. Diseases Children*, **17**: 241, 423; **18**: 107 (1919). p 371
Home, E., *Trans. Roy. Soc. London*, **A103**: 146 (1813). p. 367
Hori, T., *see* Masuda, Y.
Howe, P E., *Am. J. Diseases Children*, **21**: 57 (1921). p. 371
Howell, K M., *see* Levinson, A
Hrdina, L., *see* Schoenheimer, R.
Hueck, W, *Verhandl deut. path. Ges.*, 20 Tagung· 18 (1925). p 373
Hughes, E. H and Hart, H G, *J. Nutrition*, **9**. 311 (1935). p 352
Hunscher, H A., *see* Nims, B.
Huntsinger, M E, *see* McClure, C. W.
Hurtley, W H., *see* Garrod, A. E.
Ivy, A. C., *see* Rony, H. R.
Jakowlewa, A, *see* Wichert, M.
Jamieson, G. S., *see* Kurtz, F E.
Jones, E C, *see* Hilditch, T P.
Jost, H. and Sorg, H., *Arch ges Physiol.* (*Pflügers*), **231**: 143 (1932). p. 362
Kakiuchi, S, *Biochem. Z.*, **32**: 137 (1911). p. 372
Kerr, R. H., *see* Holmes, A. D.
King, E. J. and Dolan, M., *Biochem. J.*, **27**: 403 (1933) p 362
Knauer, H., *Jahrb. Kinderheilk*, 22 Supp. (1928). p. 354, 373
Kobert, R. and Koch, W., *Deut. med Wochschr*, **20**: 883 (1894). p 368
Koch, W, *Z physiol. Chem.*, **47**: 327 (1906). p. 353
See also Kobert, R
Koehler, A. E, *see* Hill, E
Kugler, O. E, *Am J. Physiol*, **115**: 287 (1936). p. 362
Kurtz, F. E., Jamieson, G. S. and Holm, G. E, *J. Biol Chem.*, **106**. 717 (1934). p. 354
Kusui, K., *Z physiol. Chem.*, **181**: 101 (1929). p. 362
Landenberger, L L., *see* Levinson, A.
Lasch, F., *Biochem. Z.*, **153**: 150 (1924). p. 363
Lecoq, R., *see* Leroy, A.
Le Count, E. R and Long, E. R., *J. Exptl. Med.*, **19**: 234 (1914). p. 365
Leroy, A. and associates (Lecoq, R, Veline, M., Valissant, Mme. and Barjot, G), *Lait*, **11**. 12, 144, 234, 359 (1931); *C. A.*, **25**: 5457 (1931). p. 357
Levene, P. A. and Rolf, I. P., *J. Biol. Chem.*, **46**: 193 (1921); **51**: 507 (1922). p. 361
Levinson, A., Landenberger, L. L. and Howell, K. M., *Am. J. Med Sci*, **161**: 561 (1921) p 363.
Liebermann, L., *Arch. ges. Physiol.* (*Pflügers*), **43**: 71 (1888). p. 360
Lintzel, W., *Z. Zücht, Reihe B, Tierzücht. u. Züchtungsbiol.*, **29**: 219 (1934); *C A.*, **28** 5511 (1934). p. 358
Lobstein, J. E. and Flatter, M, *Lait*, **15**: 946 (1935); *C. A.*, **30**: 1880 (1936). p. 353
Long, E. R., *see* Le Count, E. R.
Longenecker, H. E., *see* Hilditch, T. P.
Lorenz, F. W., *see* Almquist, H. J.
McCay, C. M., *see* Maynard, L. A.
McCay, C. M. and Maynard, L. A., *J. Biol. Chem.*, **92**· 273 (1931). p 356
ibid., **109**: 29 (1935). p 358
McClure, C. W, Huntsinger, M E and Fernald, A T, *Am J. Physiol*, **107**. 1 (1934) p 365
McConnell, K. P. and Sinclair, R G., *J. Biol Chem*, **118**: 123 (1937) p 352
Macheboeuf, M. and Fethke, N., *Bull. soc. chim. biol*, **14**. 507 (1932). p. 363.
McMaster, P. D., *J. Exptl. Med.*, **40**: 25 (1924). p. 364
Macy, I. G., *see* Nims, B.

Madsen, L. L., *see* Maynard, L. A.
Marsh, H. L., *see* Meigs, E. B.
Marston, H. R., *Australian J. Exptl. Biol. Med. Sci.*, 3: 217 (1926). p. 355
Masuda, Y. and Hori, T., *J. Agr. Chem. Soc. Japan*, 13: 200 (1937); *C. A.*, 31: 7548 (1937). p. 361
Maynard, L. A., *Lait*, 12: 761 (1932); *C. A.*, 27: 1665 (1933). p. 357
See also Bender, R. C., McCay, C. M.
Maynard, L. A. and McCay, C. M., *J. Nutrition*, 2: 67 (1929). p. 356
Maynard, L. A., Harrison, E. S. and McCay, C. M., *J. Biol. Chem.*, 92: 263 (1931). p. 357
Maynard, L. A., McCay, C. M. and Madsen, L. L., *J. Dairy Sci.*, 19: 49 (1936). p. 357
Meigs, E. B. and Marsh, H. L., *J. Biol Chem.*, 16: 147 (1913). p 354
Meigs, E B., Blatherwick, N. R. and Cary, C. A., *J. Biol. Chem*, 37: 1 (1919). p. 358
Mendel, L. B., *see* Read, L. L.
Mendel, L. B. and Daniels, A L., *J Biol Chem*, 13: 71 (1912). p 356
Minot, A. S., *see* Denis, W.
Miyamori, S., *Nagoya J. Med Sci*, 8: 176 (1934); *C. A*, 31: 7098 (1937) p 362
Mörner, K. A. H., *Skand. Arch. Physiol.*, 6: 332 (1895). p 372
Mortimer, B., *see* Rony, H. R
Mottram, V. H., *J. Physiol*, 47 xviii (1913). p. 361
Mühlbock, O., *Z. Kinderheilk.*, 56: 303 (1934). p.354
Müller, F., *Z. Biol.*, 20: 327 (1884). p. 367
Z. klin. Med., 12: 45 (1887). p. 367
Arch. path. Anat (Virchow's), 131, Supp., 108: 64 (1893). p.367
Munk, I., *Arch. path. Anat (Virchow's)*, 122: 302 (1890). p. 368
Nair, J. H., *see* Wiese, H. F
Nathan, M., *Arch path. Anat. (Virchow's)*, 228: 51 (1920). p. 365
Needham, J., "Chemical Embryology," Cambridge, University Press, 1931. p. 350, 351
Neuwirth, I., *see* Barbour, H. G.
Nims, B and associates (Macy, I G, Brown, M. and Hunscher, H A.), *Am. J Diseases Children*, 43: 828, 1062 (1932). p 355
Nishimoto, U., *Proc. Imp Acad (Tokyo)*, 10: 578 (1934). p 361
Oeter, H D., *see* Bürger, M
Ott, M., *see* Diemair, W
Page, I. H., *Am. J. Med. Sci*, 192: 217 (1936). p. 373
Palmer, L. S, *see* Petersen, W. E., Rimpila, C. E
Palmer, L S and Wiese, H F, *J. Dairy Sci.*, 16: 41 (1933); *C A*, 27: 1954 (1933). p 354
Pemberton, R, Cajori, F. A. and Crouter, C. Y, *Ann. Internal Med*, 2: 1243 (1929) p. 374
Petersen, W. E., Palmer, L. S. and Eckles, C. H, *Am. J Physiol*, 90: 573 (1929) p. 359
Polonovski, M., Cuvelier, L and Avenard, R, *Compt. rend. soc. biol*, 111. 6 (1932). p. 355
Pospeloff, S., *see* Wichert, M.
Pribram, E E, *Arch Gynäkol.*, 119: 57 (1923). p. 365
Ragsdale, A. C. and Brody, S., *J Dairy Sci.*, 5: 212 (1922); *C. A.*, 16: 2740 (1922). p 357
Ragsdale, A. C and Turner, C. W, *J. Dairy Sci.*, 5: 544 (1922); *C. A.*, 17: 1513 (1923). p 358
Ravdin, I S, *see* Riegel, C.
Read, L L, Mendel L B. and Vickery, H. B., *Am. J. Physiol.*, 102: 285 (1932) p 355
Reiser, R., *J Biol Chem*, 120: 625 (1937). p. 363
Rhead, A. J, *see* Hilditch, T. P.
Riegel, C, Ravdin, I S. and Rose, H. J, *Proc Soc Exptl Biol Med*, 35: 94 (1936) p. 366
Am. J. Med. Sci, 193: 446 (1937) p. 366
Riemenschneider, R W and Ellis, N R, *J Biol. Chem*, 113: 219 (1936). p. 355
Riemenschneider, R W., Ellis, N. R. and Titus, H. W, *J. Biol. Chem.*, 126: 255 (1938). p. 361
Rimpila, C E. and Palmer, L S., *J Dairy Sci.*, 18: 827 (1935); *C. A*, 30: 1133 (1936) p 354
Rohmann, F., *Arch ges Physiol. (Pflügers)*, 41: 411 (1887). p. 368
Rolf, I P., *see* Levene, P A.
Rony, H. R, Mortimer, B. and Ivy, A. C., *J. Biol. Chem.*, 96: 737 (1932). p 363, 367
ibid, 102: 161 (1933) p 363
Rose, H J, *see* Riegel, C
Rückert, W, *see* Bürger, M
Rupp, H., *see* Bickenbach, W
Russjajewa-Oparina, F., *see* Wichert, M.
Salomon, H., *Arch. Verdauungs-Krankh.*, 39: 46 (1926). p. 365
Salomon, H. and Silva, L L, *Arch. Verdauungs-Krankh.*, 36. 353 (1926). p. 365
Sano, M, *Tôhoku J Exptl. Med*, 1: 448 (1920). p. 372
Schlutz, F. W., *see* Thomas, J
Schoenheimer, R, *Z. physiol. Chem.*, 180: 1 (1929). p. 370
Schoenheimer, R and Behring, H. v, *Z. physiol Chem.*, 192: 102 (1930). p. 370
Schoenheimer, R and Hrdina, L., *Z. physiol. Chem.*, 212: 161 (1932). p. 369
Sheehy, E J, *Biochem. J*, 15: 703 (1921). p. 359
Silva, L. L., *see* Salomon, H
Sinclair, R G, *Am. J. Physiol*, 103: 73 (1933). p. 352
See also McConnell, K. P
Skarzynski, B., *Bull. intern acad. polon. sci., Classe sci math. nat.*, 1936B: II, 437. p 62
Sleightholme, J. J., *see* Hilditch, T. P.
Smith, F. H., Wells, C. A. and Ewing, P. V., Georgia Expt Sta. Bull., 122: June (1916). p 355
Sobotka, H., *see* Bloch, E.
Sorg, H., *see* Jost, H
Sperry, W. M., *J. Biol. Chem*, 68: 357 (1926) p 368
ibid., 71: 351 (1927). p. 369
ibid., 96. 759 (1932). p. 369
Sperry, W M. and Angevine, R. W, *J Biol. Chem*, 96: 769 (1932). p. 369, 370
Sperry, W. M and Bloor, W. R, *J. Biol Chem.*, 60: 261 (1924). p 368
Stevenson, S. G and Bacharach, A L., *Biochem J.*, 31: 721 (1937). p 353
Sueyoshi, Y. and Furukubo, T, *J. Biochem. (Japan)*, 13. 155 (1931). p 360
Supplee, G C., *see* Ansbacher, S.
Thannhauser, S. J., *Deut. Arch. klin. Med.*, 141: 290 (1923). p. 366
Thaysen, T. E. H., *Acta Med. Scand.*, 64: 292, 323, 354 (1926). p. 372

Thiemich, M., *Jahrb Kinderheilk*. 61. 174 (1905) p. 351
Thomas, J. and Schlutz, F. W, *Am J Diseases Children*, 56 336 (1938) p 372
Titus, H. W., *see* Riemenschneider, R W.
Turner, C. W., *see* Ragsdale, A C
Valissant, Mme., *see* Lerov, A
Veline, M., *see* Leroy, A
Vickery, H. B., *see* Read, L. L
Voit, F., *Z. Biol.*, 29: 325 (1892) p. 368
Wacker, L. and Beck, K. F., *Z. Kinderheilk.*, 29: 331 (1921). p 371
Wells, C. A., *see* Smith, F. H.
Weston, P. G., *J. Med Research*, 33: 119 (1915). p 363
Wichert, M. and Russjajewa-Oparina, F., *Arch Verdauungs-Krankh*, 34: 175 (1925). p. 365
Wichert, M., Pospeloff, S. and Jakowlewa, A., *Z. klin. Med.*, 109: 678 (1929). p. 373
Wiese, H. F., *see* Palmer, L. S
Wiese, H. F., Nair, J. H and Fleming, R. S., *Ind. Eng Chem.* (*Anal. Ed.*), 4: 362 (1932). p. 353
Wilson, K. M, *see* Boyd, E. M.
Winterseel, W, *see* Bürger, M
Wright, A., *J. Exptl. Med.*, 59: 407 (1934). p. 366
Yokoyama, Y., *Proc. Imp. Acad* (*Tokyo*), 10: 582 (1934). p. 361

Index

A

Absorption of fat, 58, 85
changes during, 98
effect on blood lipids, 136, 140, 141
cholesterol, 137
fat, 136
phospholipids, 139
esters, 86
factors affecting, 59, 141
fat-like substances, 86
form in which fat leaves intestine, 85, 91
length of intestine, 86
loops of intestine, 90
parenteral administration, 103
passage into circulation, 100
paths of, 102
phosphorylation in, 92
resynthesis, 91
soaps, 85
split products, 85
from stomach, 80
theories of, 87
transport of fat, lacteals, transfer to, 95
lymph, role of in, 97
unchanged fat, 87, 100
Acetone bodies, *see* Ketone bodies
Acid intoxication, 330
Acids, effect on blood lipids, 169
fatty, *see* Fatty acids
Acne, blood lipids in, 172
Adrenal glands, lipids of, 219
Age, effect on blood lipids, 151
Alcohols (*see also* Cholesterol, Sterols)
aminoethyl, 2
cetyl, 2
glycerol, 30
myricyl, 2
in natural combination with fatty acids, 30
straight-chain, 31
Algae, fat in, 258
Aminoethyl alcohol, 2
Aminolipids, 2
Ammonia, 36
Anemia, blood lipids in, 158
experimental, 158
human, 160
Anesthesia and narcosis, blood lipids in 156
Angina pectoris, blood lipids in, 175
Anticoagulants, effect on human blood lipids, 135
Antiketogenesis, 330
Antioxidants, 10
Apple wax, unsaponifiable of, 262
Arachidonic acid series, 24
Arteriosclerosis-atherosclerosis, 175, 223
Arthritis, blood lipids in, 177
Availability of fats, 58
Avitaminosis, effect on blood lipids, 163

B

Bacillus leprae, lipids of, 272
Bacteria, lipids of, 264
Bases in combination with fatty acids, 35
Beans, phospholipid of, 258
Beets, phospholipid of, 259
Betaine, 36
Beta oxidation, 314, 322
Bile, 364
ligation of duct on blood lipids, 182
role in intestinal digestion of fat, 84
Blood as a tissue, 115
Blood coagulation, 184
sedimentation time, 184
Blood esterase, 74
Blood lipids, 116, 186
in abnormal conditions (*see also* under individual conditions), 153, 187
acids, effect of on, 169
age, effect of on, 151
anticoagulants, effect of on human, 135
average values, different species, 122
humans, 132
carbon-dioxide combinations. 121, 186
chloride combinations, 121, 186
coagulation, 184
constants, 128
diet, effect of on, 145
diurnal variations, 134
drugs, effect of on (*see also* under individual drugs), 168
embolism, effect of on, 183
factors affecting level, 120
fat absorption, effect of on, 136
humans, 140
and gestation, 148
hemolysis, effect of on, 183
in infections (*see also* under individual infections), 172
intravenous injections, effect of on, 144
lactation, effect of on, 150
in leprosy, 175

Blood lipids (*continued*)
lipemia, effect of on, 183, 231
menstruation, effect of on, 147
in mental disease, 175
nature of, 116
normal basal levels (*see also* Absorption of fat, Postabsorptive state), 122, 135, 186
nutritional abnormalities, effect of on, (*see also* under individual abnormalities), 153
in organic diseases (*see also* under individual diseases, 175
ovulation, effect of on. 147
physico-chemical relations, 184
protein combinations, 121, 185
and puerperium, 150
reproductive cycle, effect of on. 147
in scurvy, 164
and splenectomy, 182
and tartrates, 169
in tuberculosis, 174
variations in members of same species, 129
same individual, 131
various races, 135
work, effect of on, 152
Bone marrow, lipids of, 222
human. 230
Brain and nervous system, 203
diet and brain lipids, 205
fatty acids, 204
gray and white matter, 204
lipid degeneration, 208
lipid distribution, 206

C

Cabbage. lipids in, 259
Calcium. 36
Cancer, blood lipids in (*see also* Tumors), 162
Carbohydrates, transformation to fat, 237
change of fat to, 302
Carbon dioxide-lipid combinations. 186
Carbon dioxide solubility. effect of lipids on, 186
Carotene, 7
Castration. blood lipids in, 182
Catabolism of fats, 313
Cephalin, 2. 16
composition, 17
preparation, 17
properties. 14. 15, 16
Cerebrosides (glycosides), 2, 19
determination, 49
Cerebrospinal fluid, 363
Cetyl alcohol, 2
Chaulmoogric acid. 25
Chloride-lipid combinations. 186
Choleic acid principle. 107
Cholesterol, absorption, 104
in blood, *see* Blood lipids
chemical and physical properties, 35
determination, 42, 48, 50
digestion and absorption, 104
in eggs, 362
melting point, 35
metabolism of, 336
in microorganisms, 264
in milk, 353
occurrence, 32
and phospholipids, 36, 246
physiology of, 34, 244
properties of, 35
role in metabolism, 313
structure of, 2, 33
in tissues, *see* Tissue lipids
Cholesterol esterase, 76
Cholesterol esters, 11
determination, 43, 48
Cholesterol gout, 232
Choline, 2
in fatty livers, 217
Chyle, lipids in, 363
Chylomicrons, 136, 140
Classification of lipids, 1
Clupanodonic acid, 24
Coagulation of blood, 184
Cold-blooded animals, lipids of, 228
Compound lipids. 12
Constants, lipids of, 128
Constitution of fats. 3
Corn endosperm, unsaponifiable of. 260
Corn oil, unsaponifiable of, 260
Corn pollen, unsaponifiable of, 260
Corpuscles, lipids of, 128, 186, 187
Corpus luteum, lipids of, 223, 251
Cottonseed oil, unsaponifiable of, 261
Cyclic acids, saturated, 21
unsaturated, 25
Cysticercus fasciolaris, lipids of, 229
Cysts, lipids of, 234

D

Deficiency of fat, 318
Dehydration of fats, 38
Dehydrogenation of fatty acids, 28
Deposition and mobilization of fats, 294
Derived lipids, 2, 21
Desaturation of fatty acids in liver, 314
Determination of lipids, *see* Methods for determination of lipids
Deuterium as a measure of fat metabolism, 191
Deuterium as a measure of sterol metabolism, 293
Diabetes, blood lipids in, 164
experimental, 166
Dicarboxylic acids, 321
Diet, effect on blood lipids, 143
effect on brain lipids, 205

Diet (*continued*)
effect on feces lipids, 368
effect on stored fat, 197, 236
Digestion of fat (*see also* under individual lipids), 58, 78
absorption from stomach, 80
bile, role of in, 84
enzyme action, 83
intestine, 81
factors in fat splitting, 79
melting point, effect of on, 59
nature of fatty acids, effect of on, 61
pancreas in, 83
passage from stomach, 81
species variation, 62
stomach, 79
temperature, effect of on, 61
vitamins, effect of on, 62
Diurnal variations, blood lipids, 134
Double bonds, position in fatty acid, 23
Drugs, effect on blood lipids, 168
Dystrophy, nutritional, effect on muscle lipids, 203

E

Eczema, effect of on blood lipids, 171
Eggs, 359
cholesterol in, 362
fat metabolism in, 337
incubation, changes during, 362
lipids of, 360
phospholipids in, 361
unsaturation of fatty acids of, 361
Elaidin, feeding of, 94, 294
Element constant, 249, 253
Embolism, fat, effect on blood lipids, 183
Embryo, food of, 350, 359
fat metabolism in, 337
Emotional stimulation, effect on blood lipids, 183
Energy production, role of fat in, 310
Enteric bacilli, lipids in, 273
Enzymes, lipid-splitting, 62
action on intestinal digestion of fat, 83
Epihydrin aldehyde, 10
Epilepsy, blood lipids in, 170
Essential fatty acids, 318
eczema, 171
Esterases, 63, 73
blood, 74
cholesterol esterase, 76
function, 77
liver, 64, 75
occurrence, 74
origin, 73
tissues, 75
Excretions, 366
ketone bodies of, 335
Eyes, lipids of lenses, 227

F

Fasting and blood lipids, 154
and muscle lipids, 202
Fats, 1, 3
absorption, *see* Absorption of fat
availability, 58
blood, *see* Blood lipids
Brain, 204
catabolism, 313
change to carbohydrate, 302
chemical properties, 8
constitution, 3
deficiency, 318
dehydration, 38
deposition and mobilization, 294
determination, 43, 49
digestion of, *see* Digestion of fat
effect of temperature on, 61
energy production, role in, 310
extraction of, 38
formation from carbohydrate, 237
formation from food fat, 238
formation from protein, 236
fruit-coat, 262
functions, 310
hen's egg, 337, 359
high-fat diet, effect of on, 336
human, 228
hydrolysis, 8
intravenous, 144
length of chain, 61
mammal, 340
marine, 4
melting point and digestibility, 5, 59
metabolism, 286
in embryo, 337
nutritional availability of, 58
optical properties, 8
ox depot, 4
oxidation catalysis, role of fat in, 311
parenteral administration of, 103
passage through intestinal mucosa, 85
phosphorylation of, 92
in pigments, 7
in plants, 4
environmental effects, 241, 242
storage in, 242
properties of, 4
resynthesis of, 91
in seeds, 4
solubility of, 4
spectroscopic behavior of, 8
in starch, 257
stored, 4, 196, 236
diet, effect of on, 197, 236
location, effect of on, 240
temperature, effect of on, 240
synthesis of, 10
intestinal, 91
tissues, nature and sources (*see also* Tissue lipids), 236

Fats (*continued*)
transformation to carbohydrate, 302
transportation, effect on blood lipids, 298
glycogen-fat balance in, 297
intestinal walls, 95
leucocytes, 96
lymph, 97
mobilization to liver, 294
thoracic duct, 100
utilization, effect of species on, 62
Fatty acids, 21
alcohols in natural combination with, 30
bases combined with, 35
in blood, *see Blood* lipids
in blood phospholipids, 117
in brain lipids, 204
dehydrogenation, 28, 314
desaturation of in liver, 314
determination, 43, 47, 51
dicarboxylic, 321
distribution and occurrence, 22
double bonds, position in, 23
in eggs, unsaturation of, 361
essential, 318
in feces, 366, 371
halogen absorption, 28, 49
hydrogenation, 27, 317
identification, 43
isomerism, 22
ketone bodies, production from, 304
in lecithin, 14
melting point, 26
in milk, 353
natural occurrence, 22
nature of, in digestion and absorption of fat, 61
occurrence, saturated, 21
unsaturated, 21
oxidation, 26
and carbohydrates, 330
beta, 314, 322
in vivo, 324
at double bond, 320
multiple, 314, 325
peroxides, 9
in phospholipids, 117, 252
properties, 4, 6, 25
relative values in nutrition, 320
saturated, 21
saturation, 317
soap formation, 28
solubility, 4, 25
surface properties, 28
unsaturated (*see also* Unsaturated fatty acids), 21
utilization, nature of on, 61
Feathers, lipids of, 226
Feces, 366
effect of diet on lipids, 368
Feces (*continued*)
fatty acids of, 366, 371
lipids of, 366
sterols of, 366, 371
Fetus, lipids in, 224, 340
Food fat, formation of fat from, 238
variation of phospholipids with, 254
Fruits, lipids of, 259

G

Galactolipids, 19
Gall bladder, lipids of, 228
Gastric lipase, 66
Gastric mucosa, lipids of, 227
Gaucher's disease, 170, 232
Gestation and blood lipids, 148
Glucose, oxidation of ketone bodies with, 330
Glycerides, 3
synthesis of, 10
Glycerol, 30
Glycogen-fat balance in liver, 297
Glycolipids, 2, 19
Grains, lipids of, 260
Grass, unsaponifiable of, 262
Grasshopper, eggs, lipids of, 229
muscle, lipids of, 229
Green leaves, unsaponifiable of, 262
Gums, lipids of, 227

H

Hair, lipids of, 226
Halogen absorption of fatty acids, 28, 49
Heart, lipids of, 199, 200
Hemolysis, effect on blood lipids, 183
Hepatectomy, effect on blood lipids, 182
High-fat diet, adaptation to, 333
effect on metabolism, 336
Hormonal regulation of ketone bodies, 329
Human fat, lipids of, 228
Hydnocarpic acid, 25
Hydrazine poisoning, effect on blood lipids, 169
Hydrocarbons, 36
Hydrogenation of fatty acids, 27, 317
Hydrolysis, of cephalin, 15
of fats, 8, 38
of lecithin, 15
Hydrolysis-resynthesis theory, 97
Hydroxy acids, unsaturated, 25
Hypertension, effect on blood lipids, 175
Hypertrophy and muscle lipids, 256
Hypophysis dysfunction, effect on blood lipids, 182

I

Identification of fatty acids, 43
Immunity, effect on blood lipids, 172
  on liver lipids, 215
Incubation of eggs, changes during, 362
Infections, blood lipids in, 172
  effect on liver lipids, 214
Insulin, effect of, on blood lipids, 167
    on ketone bodies, 333
    on liver lipids, 214
Intestinal lipase, 70
Intestinal mucosa, passage of fat through, 85
Intestine, digestion of fat in, 81
  form in which fat leaves, 85, 91
  length of, 86
  lipids of, 227
  loops of, 90
  synthesis of fat in, 91
  walls of, fat transport in, 95
Intravenous injections, effect on blood lipids, 144
Iodine number, determination, 49
Irradiation, effect on blood lipids, 164
Isomerism, fatty acid, 2

K

Ketogenesis, 326
Ketone bodies, 326
  effect of insulin on, 333
  formation of, 327
  hormonal regulation, 329
  neutralization and excretion, 335
  oxidation with glucose, 330
  production from fatty acids, 304
  from proteins, 328
Ketosis, 327, 330
  adaptation to, 333
  effect of insulin on, 333
Kidney, disorders and blood lipids, 179
  lipids in, 219
    human, 230
Kreis test, 10

L

Lactation cycle, 357
Lactation, effect on blood lipids, 150
Lacteals, transfer of fat to, 95
Lecithin, 2, 12
  in blood, *see* Blood lipids
  fatty acids in, 14
  hydrolysis, 15
  preparation, 14
  properties, 15
  structure, 13
  surface films of, 16
  synthesis, 15
  switterion structure, 13, 16
Lecithinases, 76
Lecitho-proteins, 185
Leprosy, blood lipids in, 175
  lipids of bacilli, 272
  skin nodules of, 231
Leucocytes, transfer of fat by, 96
Liebermann-Burchard reaction, 51
Ligation of bile duct on blood lipids, 182
Lignoceryl sphingosine, 36
Linoleic acid series, 23
Linolenic acid series, 24
Linseed oil, unsaponifiable of, 261
Lipases, 8, 66, 82
  gastric, 66
  intestinal, 70
  lower organisms, 71, 72
  pancreatic, 67, 83
  plant, 70
  ricinus, 8, 70
  from seeds, 8, 70
  stomach, 79
  testing and measuring, 72
Lipemia, effect of on blood lipids, 183, 231
Lipids, balance, 120
  in blood, *see* Blood lipids
  compound, 2, 12
  constants, 128
  corpuscles of, 128, 186, 187
  derived, 2, 21
  determination, methods of, 37
  diurnal variations, 134
  effect of fat absorption, *see* Fat absorption
  extraction, 38
  in feces, 366
  in fetus, 224, 340
  in fruits, 259
  human, 131
  lipid-protein, 121
  liver, *see* Liver lipids
  in lungs, 198, 199
  in lymph, 363
  macromethods, 37
  metabolism, 286, 294, 302, 337
  metabolic and functional, 196
  methods of study, 37
  micromethods, 44
  of microörganisms, 263
  mobilization and storage in liver, 294
  in molds, 275
  in muscle, 198, 202, 203, 230, 250, 256
  nomenclature of, 1
  normal level, 122
  normal variations, 129, 131
  physico-chemical relations of, 36, 184
  of placenta, 224, 341
  plants, 4, 241, 257
  plasma, 186, 187
  platelets, 128
  postabsorptive, 118
  and proteins, 121, 185

Lipids (*continued*)
racial differences, 135
removal mechanism, 121
in reproductive organs, 147
salmon, migrating, changes in, 309
of secretions, 350
simple, 1, 3
of skin, 225, 373
of spleen, 221, 230
split-products, 36
in starches, 257
synthesis of, 10, 91, 287
in tissues, *see* Tissue lipids
in urine, 372
Lipins, 1
Lipoid, 1
Liver, desaturation by, 217
disease, effect on blood lipids, 177
esterase, 64, 75
fatty livers, 215, 291
choline in, 217
mobilization of fat to, 290
phospholipids of, role in metabolism, 300
Liver lipids, 198, 199, 208, 230
effects of various factors on, 213
immunization, 215
infections, 214
insulin, 214
thyroxin, 214
Location, effect on stored fat, 240
Lungs, effect on blood lipids, 142
lipids of, 198, 199
human, 230
Lymph, lipids in, 363
role of in fat transport, 97
Lysocephalin, 17
Lysolecithin, 17

M

Magesium, 36
deficiency and blood lipids, 164
Melting point, cholesterol, 35
fat, 5, 59
fatty acids, 26
factor influencing digestion and absorption, 59
Menstruation, effect of on blood lipids, 147
Mental disease, blood lipids in, 175
Metabolic excesses, blood lipids in, 169
Metabolism of lipids, 286
in eggs, 337
in embryo, 337
intermediary, 294
radioactive tracers in, 294, 302
Metabolism, role of phospholipids in, 300, 312
Methods for determination of lipids, 37
macromethods, 37
cholesterol, 42
Methods for determination of lipids (*continued*)
macromethods (*continued*)
cholesterol esters, 43
dehydration and extraction, 38, 41
fats, 43
fatty acids, 43
hydrolysis, 38
phospholipids, 42
separation, 40, 42
micromethods, 44
cerebrosides, 49
cholesterol, 48, 50
cholesterol esters, 48
extraction, 45
iodine number, 49
neutral fat, 49
phospholipids, 46
sphingomyelin, 49
total fatty acids, 47, 51
Meyer-Overton theory, 156
Microorganisms, lipids of, 263
Milk, 352
composition, 353
human, 355
fat particles, coat of, 354
fatty acids, 353
origin, 355
phospholipid and cholesterol, 353
pigeon, 355
timing of secretion, 359
Mitochondria, nature of, 244
Molds, lipids in, 275
Muscle lipids, 198, 250
fasting, 202
human, 230
hypertrophy, 256
injection of thyroidin and thyroxin, 203
nutritional dystrophy, 203
normal variations, 198
relation to physiological activity, 256
Muscular dystrophy, 170, 203
Myricyl alcohol, 2

N

Nephritis, blood lipids in, 179
Nephrosis, blood lipids in, 180
Neutral fat, from carbohydrate, 288
from protein, 287
oxidation mechanisms, 313
Niemann-Pick's disease, 170, 231, 232
Nutritional dystrophy, effect on muscle lipids, 203
Nutritional state, effect on blood lipids, 143
Nutrition, relative value of fatty acids in, 320

O

Obesity, 170, 308
Oils, 1, 3,
Oleic acid series, 23
Optical activity of fats, 8
Organic diseases, blood lipids in, 175
Ovary, lipids in, 222
Ovulation, effect on blood lipids, 147
Ox depot fat, 4
Oxidation, catalysis, role of fat in, 311
  of fatty acids, 26, 314, 326
    beta, 314, 322
    and carbohydrates, 330
    at double bond, 320
    multiple, 314, 325
    positions other than beta, 323
  of ketone bodies with glucose, 330
  mechanisms for neutral fat, 313
Oxynervonic acid, 25

P

Pancreas, effect on digestion of fat, 83
Pancreatic lipase, 67, 83
  preparation, 69
  properties, 69
Pancreatitis and blood lipids, 181
Peroxides, 9
Phlorizin effect of on blood lipids, 168
Phosphatidates, 2, 19
Phosphatides (*see also* Phospholipids), 2
Phospholipid: cholesterol ratio, 36, 246
Phospholipids (*see also* individual ones), 2, 3, 12
  absorption of, 103
  in beans, 258
  in beets, 259
  in blood, *see* Blood lipids
  —cholesterol balance, 36, 246
  determination, 42, 46
  digestion of, 103
  in eggs, 361
  fatty acids in, 117, 252
  in milk, 353
  in plants, 258
  in potatoes, 259
  in pregnancy, 251
  preparation, 14, 15, 42
  physiology of, 244
  in rape seed, 259
  role in metabolism, 300, 312
  synthesis of, 293
  in tissues, *see* Tissue lipids
  variation with food fat, 254
Phosphorus, effect of on blood lipids, 168
Phosphorylation of fats, 92
Phthioic acid, 21
Physico-chemical relations of lipids, 36, 184
Pigeon milk, 355
Pigments, fat, 7
Pine tips, unsaponifiable of, 263
Placenta, lipids of, 224
  passage of lipids through, 224, 341, 351
Plankton, fat in, 258
Plant lipases, 8, 70
Plants, lipids of, 4, 241, 242, 257
Plasma, lipids of, 186, 187
Platelets, lipids of, 128
Pollen, corn, fat in, 259
Postabsorptive state, 118
Potatoes, phospholipids in, 259
Pregnancy, phospholipids in, 251
Preparation of phospholipids, 14, 42
  cephalin, 17
  lecithin, 14
Proteins, combinations with lipids, 121, 185
  ketone bodies from, 328
  transformation to fat, 236, 287
Puerperium and blood lipids, 150

R

Racial differences, lipids, 135
Radioactive elements as tracers, 94, 95, 294
Rancidity, 9
Rape seed, phospholipids in, 259
Reproductive cycle, effect on blood lipids, 147
Reproductive organs, lipids of, 222
Resynthesis of fat, 91
Rice embryo, unsaponifiable of, 263
Ricinoleic acid, 25
Ricinus lipase, 8, 70

S

Salmon, lipid changes during migration, 309
Saturated acids, 21
Saturation of fatty acids, 317
Scurvy, blood lipids in, 164
Schüller-Christian disease, lipids in, 232
Secretions, lipids of, 350
Sedimentation time of corpuscles, effect on blood lipids, 184
Seed fat, 4
Serological reactions and blood lipids, 173
Simple lipids, 1, 3
Skin, lipids of, 225
  lipids excreted through, 373
Snake root, effect on blood lipids, 169
Soaps, 28, 61, 85
"Soft pork" problem, 238
Solubility of, carbon dioxide, effect of lipids on, 186

Solubility of (*continued*)
cephalin, 14, 15, 16
cerebrosides 20
cholesterol, 35
fat, 4
fatty acids, 4, 25
glycerol, 30
lecithin, 14, 15
sphingomyelin, 18
Soy bean, unsaponifiable of, 262
Species variation, effect on blood lipid values, 122
effect on digestion and absorption of fats, 62
Spectroscopic behavior of fats, 8
Sphingomyelin, 2, 18
in blood, *see* Blood lipids
composition and structure, 18
determination, 49
Niemann-Pick's disease, 232
properties, 18
Sphingosine, 2, 18, 35, 36
Spleen, lipids of, 221
Human, 230
Splenectomy, effect of on blood lipids, 182
Split-products, 36
absorption of, 85
Squalene, 2, 36
Starches, lipids of, 257
Steatorrhea, 372
Sterols, 31, 104
absorption of in animal body, 104, 108
and fat metabolism, 33, 105
of feces, 366, 371
metabolism of, as measured by deuterium, 293
role as essential cellular constituents, 244
synthesis, 291
Stomach, absorption of fat from, 80
digestion of fat in, 79
lipase, 79
Straight-chain acids, 21
Straight-chain alcohols, 31
Structure of, bases in combination with fatty acids, 35
carotene, 7
cerebrosides, 20
cholesterol, 33
fats, 3
glycerol, 30
lecithin, 13
sphingomyelin, 18
sterols and related compounds, 32
Sulfolipids, 20
Surface films of lecithin, 16
Surface properties of fatty acids, 28
Sympathectomy, effect on blood lipids, 183
Synthesis of lipids, 10, 91, 287
of glycerides, 10
Synthesis, of lipids (*continued*)
intestinal, 91
of lecithin, 10
of neutral fat, 10, 287
from carbohydrate, 288
from protein, 287
of sterols, 291
of phospholipids, 293
Syphilis, blood lipids in, 175

T

Tartrates, effect of on blood lipids, 169
Temperature, effect on digestion and absorption of fat, 61
effect on stored fat, 240
Thoracic duct lymph, 100
Thyroid conditions and blood lipids, 181
Thyroidin, effect on muscle lipids, 203
Thyroxin, effect on liver lipids, 214
effect on muscle lipids, 203
Timothy bacillus, lipids of, 267
Tissue esterases, 75
Tissue lipids, 196
abnormal conditions (*see also* under individual conditions), 202, 229
diseases involving lipid deposits, 231
functions, 310
energy production, 310
oxidation catalysis, 311
nature (*see also* under individual lipids), 236
normal (*see also* under individual tissue or organ), 198
Tobacco seeds, unsaponifiable of, 262
Transudates and exudates, 363
Tributyrin, 60
Tubercle bacillus, lipids of, 14, 268
Tuberculosis, blood lipids in, 174
Tuberculostearic acid, 21
Tumors, lipids of, 234, 251
Tung oil, 23
Typhoid bacillus, lipids in, 273

U

Unsaponifiable of, apple wax, 262
blood, *see* Blood lipids
corn endosperm and corn pollen, 260
corn oil, 261
cottonseed oil, 261
grass, 262
green leaves, 262
linseed oil, 261
pine tips, 263
rice embryo, 263
soy bean, 262
tobacco seeds, 262
wheat, 262
Unsaturated fatty acids, 21
arachidonic series, 24
cyclic, 25

Unsaturated fatty acids (*continued*)
  double bond, position of, 23
  hydroxy, 25
  linoleic series, 23
  linolenic series, 24
  liver, role of, 314
  need of by organism, 317
  oleic series, 23
  structure of, 23
Unsaturation, in eczema, 171
Urine, lipids of, 372
Utilization of fatty acids, effect of nature on, 61

V

Villi, transfer of fat by, 95
Vitamins, effect on fat utilization, 62, 163, 342
Vitamin A, 164, 342
  destruction of, 9
Vitamin B, 62, 163, 342
Vitamin C, 164, 343
Vitamin D, 342
Vitamin G, 343

W

Waxes, 2, 11
  apple cuticle, 262
  grasses, 262
Wheat, unsaponifiable of, 262
Wood, fat in, 260
Wool, lipids of, 226
Work, effect on blood lipids, 152

X

Xanthoma, lipids of, 230
Xanthomatosis and blood lipids, 169

Y

Yeast, lipids of, 273
Yellow fever, lipids of tissues of monkeys, 231

Z

Zwitterion, 13, 16